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(57) Abstract

The application describes a novel mechanism of action, that is modulation of the specific effectiveness of I-kappa-kinases or cyclic nucleotide phosphodiesterases (PDEs) which have the ability to cleave cGMP or cAMP. The preferred mode of action is dislocation, disruption of targeting or interference with redistribution of specific isoforms or splice variants of PDE4, PDE5, or I-kappa-kinases from their anchoring sites within cells, thereby modulating their specific effectiveness, not their enzymatic capacity. The chemical entities may be useful in preventing or treating in an animal, preferably a human, in need thereof an adverse condition which may be reduced or abolished by modulating the specific effectiveness of PDE4, PDE5, or I-kappa-kinases.

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SPECIFIC THERAPEUTIC INTERVENTIONS OBTAINED BY INTERFERENCE WITH REDISTRIBUTION AND/OR TARGETTING.

SUMMARY OF THE INVENTION

This application describes a novel mechanism of action of chemical entities in order to prevent or treat adverse conditions which may be reduced or abolished by modulating the effectiveness of I-kappaB kinase (IKK) or cyclic nucleotide phosphodiesterases (PDE:s) by modulation of their targeting or localisation in the cell. The preferred mode of action being sought is dislocation or interference with the targeting of specific isoforms of IKK or PDE:s and interference with their anchoring sites within cells, thereby reducing their specific effectiveness, not directly their enzymatic capacity.

In its broadest aspect, the present application relates to a novel method for preventing or treating, in an animal in need thereof, an adverse condition which may be reduced or abolished by modulating the activity of one or more IKKs or PDE:s having the ability to cleave cAMP or cGMP. The method comprises modulation of the specific effectiveness of IKKs or PDE:s by modulating their spatial distribution within cells of the animal. The IKK is chosen from the group consisting of IKKα, IKKβ, IKKγ and NIK. In one embodiment IKKβ is the preferred isoform. The PDE:s are chosen from the group consisting of PDE1, PDE2, PDE3, PDE4, PDE 5, PDE6, PDE7, PDE8, PDE9 and PDE10. More specifically, the method relates to PDE4 and isoforms thereof, such as PDE4D, and splice variants of PDE4D, such as PDE4D1, PDE4D2, PDE4D3, PDE4D4 and PDE4D5. The animal with the adverse condition may be a mammal and preferably a human.

In one embodiment of the invention modulation of the specific effectiveness of the PDE is a dislocation of the PDE from a native location within the cell.

In another embodiment of the invention modulation of the specific effectiveness of the PDE involves a disruption of its targeting to a native location within the cell.

In another embodiment of the invention modulation of the specific effectiveness of the PDE involves interference with the redistribution of the PDE, the redistribution being associated with an increase or a decrease of the specific effectiveness of the PDE. The modulation of the specific effectiveness of the PDE may involve both an up-regulation or a down-regulation of the effectiveness of the PDE to perform its function within the cell.

The present invention provides compositions and methods for modifying the activation of NF-kappaB by mis-targeting and/or modulation of the redistribution of specific IKKs.

In one embodiment we specifically modulate the targeting of IKKβ. We have developed two molecular probes PS473 and PS474 that upon expression in a relevant cell system will dislocate endogenous IKKβ from its anchoring site. The mis-targeting has, as shown in example 1, significant functional consequences that can be related to a diminished ability of cytokines and other stimuli to activate NFkappaB. We thus show that IL-1 induced translocation of NFkappaB from cytoplasm to the nucleus is effectively inhibited, and furthermore as a consequence thereof we found that NFkappaB-induced transcriptional activation was inhibited.

NFkappaB has been shown to rescue transformed cells from undergoing apoptosis when exposed to pro-inflammatory cytokines like TNFα (Baichwal, V.R. & Baeuerle, P.A. (1997) Curr Biol 7, R94-6). To substantiate that mis-targeting of IKKβ is an effective way of blocking the functional effect of IKKβ, we analysed whether PS473 was able to influence TNFα-induced apoptosis. As seen in example 1 the probe (PS473) was found to hypersensitise cells to apoptotic stimuli.

In another embodiment the present invention provides agents that modulate the targeting and/or redistribution of IKKs. Such agents include polypeptides that comprise a putative leucine zipper region of IKKβ. Included are DNA molecules and expression vectors that encode for the described peptides, furthermore host cells are provided that express said peptides in a stable or transient expression system.

In another embodiment the invention provides a method for finding compounds that modulate targeting and redistribution of IKKβ and of derivatives thereof. The method renders itself to screening for compounds that modulate the functional activity of I-kappaB kinase β through modulation of one or more of multiple targeting sites of IKKβ (or other IKKs) and which thereby cause either a partial or a complete inhibition of the NF-kappaB activation. The method will allow for identification of compounds that modulate said targeting or redistribution in specific cell types.

The presented novel mechanism of action will be useful in the treatment of the following diseases/conditions: chronic inflammation, asthma and chronic bronchial hyperreactivity

of non-asthma etiology, rheumatoid arthritis and pelvospondylitis, ulcerative colitis and Crohn's disease, diabetes mellitus type I, systemic lupus erythematosus, myasthenia gravis, Hashimoto's thyreoiditis, Graves' disease and immune thrombocytopenic purpura, acute respiratory distress syndrome (ARDS) and septic shock as well as depression.

Background

Chronic inflammation is the result of unbalanced and continued production of inflammatory cytokines. Cytokines are produced in cascades, the pro-inflammatory TNFα and IL-1β often responsible for initiating a process, which leads to a more general production of further cytokines. This cascade of gene expression is largely under the control of NF-kappaB, a ubiquitous transcription factor that, by regulating the expression of multiple inflammatory and immune genes, plays a critical role in host defence and in chronic inflammatory diseases (Sen and Baltimore, 1986; Mukaida *et al.*, 1990; Beg *et al.*, 1993; Cogswell *et al.*, 1993). NF-kappaB is activated not only by cytokines, but also by reactive oxygen species (ROS), viruses, and a range of other generally noxious and pathogenic stimuli (Blackwell *et al.*, 1997; Schulzwe-Osthoff *et al.*, 1997). Activation of NF-kappaB via ROS has been implicated in neurodegenerative disorders such as Parkinson's and Alzheimer's (Lesoualc'h *et al.*, 1998; O'Neill *et al.*, 1997) and also in inflammatory bowel disease (Jourd'heuil *et al.*, 1997). Tissue inflammatory reponse to x-rays is mediated directly by NF-kappaB (Hallahan *et al.*, 1995). Activation of NF-kappaB has been implicated in the production of atherosclerotic lesions of smooth muscle cells (Bourcier *et al.*, 1997) and in cardiac inflammatory disorders (Hattori *et al.*, 1997). NF-kappaB/Rel transcription factors are also known to play a role in the pathogenesis of certain tumours, especially those of haematopoetic origin (Neumann *et al.*, 1997), and constitutive (autocrine) activation of NF-kappaB is known to promote a resistance to apoptotic stimuli (Giri *et al.*, 1998). Inhibitors of NF-kappaB should increase the cytotoxic efficacy of anticancer chemotherapies (Bours *et al.*, 1998).

The inflammatory pathways are notoriously complex, yet the feasibility of reducing or eliminating inflammatory responses through modulation of NF-kappaB activity has already been demonstrated in a number of different cells (Makarov *et al.*, 1997).

The NF-kappaB/Rel group of transcription activators and their co-evolved regulatory proteins, the inhibitors of kappa B (I-kappaBs), play important roles in many cellular

signalling processes in vertebrates, which include controlling communication between cells, embryo development, maintenance of cell type specific expression of genes as well as co-ordinating the inflammatory response to stressors and viral infection (Wulczyn *et al.*, 1996). The key proteins involved in this control system divide into distinct groups: a) Those that bind DNA. These belong to the Rel family of transcription factors (Ghosh *et al.*, 1990) and include p50, p65, p52/49, p75/Rel and RelB. Only dimers bind DNA, but these can be homodimers or heterodimers. p65/p50 heterodimer is the most abundant, and plays a more elaborate role than other factors in regulating gene expression (Baldwin, 1996). b) Those that interact with the DNA-binding subunits in cytoplasm, which include the inhibitory I-kappaBα and I-kappaBβ molecules (Bauerle and Baltimore, 1988), and the precursor molecule p105 (Naumann *et al.*, 1993). c) Those transcriptional coactivators which interact with the DNA-binding subunits in the nucleus, such as Bcl3 (Nolan *et al.*, 1993; Watanabe *et al.*, 1997) and Cbp/p300 (Zhong *et al.*,, 1998). d) Kinases which activate proteasomal destruction of I-kappaBα and β subunits - the I-kappaB kinases (Beg *et al.*, 1993). e) Kinases which directly phosphorylate the DNA-binding subunits in cytoplasm and nucleus to modulate their activity, such as PKA (Zhong *et al.*, 1998), casein kinase II (Bird *et al.*, 1997) and others (Hayashi *et al.*, 1993; Schulze-Osthoff *et al.*, 1997).

Inactive p65/p50 NF-kappaB dimers are held in the cytoplasm coupled to inhibitory I-kappaB molecules (α and β isoforms) via the p65 subunits. Activated I-kappaB kinases (IKK) phosphorylate the inhibitors, targeting them for ubiquitination and subsequent proteasomal digestion (Beg *et al.*, 1993). The released subunits translocate to the nucleus and there activate transcription.

The I-kappa kinases (IKK-α, IKK-β and IKK-γ) have been shown to be part of a large multi-component complex (Chen et *al.* 1996; Rothwarf et *al.*, 1998). It is likely to assume that the assembly and disassembly of the IKK complex is controlled by a scaffold protein termed IKK-complex-associated protein, IKAP (Cohen et *al.* 1998). It is expected that a tight assembly of the complex is necessary for the IKKs to be activated by the NF-kappa-B-inducing kinase (NIK) and thereby induce phosphorylation of the I-kappaB subunits. Interestingly the affinity of IKK-β for IKAP diminishes upon phosphorylation of IKK-β by NIK.

Glucocorticoids (GC) are powerfully efficient modulators of inflammation, but suffer from the potential hazards of suppressing necessary protective responses to infection and

decreasing some essential healing processes. They modulate cytokine expression by a combination of genomic mechanisms. The activated GC-receptor complex can (i) bind to and inactivate AP-1 or NF-kappaB, (ii) upregulate I-kappaB production via GC response elements (iii) reduce the half-life of cytokine mRNAs (Brattsand & Linden 1996). But steroid treatment broadly attenuates all cytokine production from all lymphocytes, so not only do levels of the inflammatory cytokines fall, but also that of the anti-inflammatory IL-10. Specific modulation of Th1-type pathways would be an initial goal of this project. It is also known that some fibroblast cell NF-kappaB-mediated responses are likely governors of inflammatory progression, so inhibition of such responses could have detrimental effects (Smith et *al.*, 1997). Therapies, which maintain appropriate feedback systems, but modulate inappropriate cytokine production represent an unmet medical need.

An attractive therapeutic intervention to be used in the treatment of chronic inflammatory conditions is inhibition of the I-kappaB degradation. Blocking the ubiquitin proteasome pathway (PharmaProjects, Accession no. 023654 and 027675), can directly inhibit this degradation. Another mechanism that is being pursued is inhibition of the enzymatic activity of either of the IKKs or NIK (public statement from Signal Pharmaceuticals).

Very many extracellular signals are transduced via intracellular systems employing the cyclic nucleotides cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) as intermediaries, or second messengers. The processes mediated by cAMP and cGMP include control of smooth muscle tone, learning, vision, cellular differentiation, control of pro-inflammatory mediator production and action, apoptosis, lipogenesis, glycogenolysis and gluconeogenesis, circadian rhythms, cardiac function, and mood control through noradrenergic potentiation.
Cyclic nucleotides are generated by adenylate and guanylate cyclases (ACs and GCs, respectively) from ATP and GTP, signal to cAMP- and cGMP-dependent effector proteins such as protein kinases (cAKs and cGKs, respectively) and certain ion channels. cAMP and cGMP are removed by phosphodiesterases (PDE:s). The required specificity of signals generated by these systems arises from diversity of type, tissue-specific expression and intracellular placement of the enzymes involved. For instance there are nine isoforms of ACs known plus additional splice variants, soluble and membrane located forms of GCs, multiple isoforms of the cAK and cGK kinases, and very many isoforms of PDE:s of which over 30 have been identified (Perry and Higgs, 1998; Houslay and Milligan, 1997; Beavo, 1995). Additional specificity arises from

targeting particular signalling enzymes to restricted locations within cells; this is the function of scaffold and anchoring proteins, such as the AKAP family, and not only may they place enzymes close to their substrates, but they may also serve to recruit multiple enzymes into functional signalling units (Pawson and Scott, 1997).

Inactivation of cAMP/cGMP occurs by hydrolysis of the 3'-ester bond, catalysed by the PDEs. The PDE:s are key components of the cyclic nucleotide signalling systems, allowing local concentration differences of the cyclic nucleotide messengers to be established, between adjacent tissues, between adjacent cells, even within a single cell between different volumes of cytoplasm. The ability to generate such heterogeneity in the distribution of concentrations of a commonly shared signalling molecule, such as cAMP, is at the heart of directed signalling processes. To be of therapeutic value, cyclic nucleotide control has to be achieved with defined cellular selectivity (Perry and Higgs, 1998). It is the therapeutic opportunities offered by certain of the PDE:s that concerns this application.

Ten families of PDE:s have been identified, designated simply PDE1 to PDE10. Within each family there are two or more related but distinct gene products (A, B, C, etc.) and for each of these alternative mRNA processing gives rise to multiple splice variants, identified by an additional arabic numeral in accordance with the most recent nomenclature recommendation (Molecular Pharmacology 46:399-405, 1994). All PDE gene products identified so far have two functional domains per molecule, one catalytic, and one regulatory. The catalytic domain lies towards the carboxylic acid terminus of each PDE protein and has the greatest homology between the PDE families, being >75% homologous at the amino acid level (Perry and Higgs, 1998). Nevertheless, each of the more than 30 PDE:s known have individually distinct substrate specificities, kinetic characteristics, regulatory properties and cellular and subcellular distributions (Houslay and Milligan, 1997).

PDE:s 4, 7 and 8 are highly specific for cAMP. PDE:s 5, 6, 9 and 10 are selective for cGMP. PDE3s bind cAMP and cGMP with similar affinity, but hydrolyse cAMP most efficiently, cGMP rather poorly. PDE3s are therefore negatively regulated in their cAMP hydrolysing ability by cGMP. PDE:s 1 and 2 hydrolyse both cAMP and cGMP, but with PDE1 the relative efficiencies vary with isoenzyme subtype (Perry and Higgs, 1998).

The amino terminal ends of PDE:s consist of the regulatory domains, which are very different both between families and between variants within families. This region contains variously: a binding domain for Ca^{2+}-calmodulin (CaM) in PDE1; non-catalytic cGMP-binding sites in PDE:s 2, 5 and 6; a binding domain for the signalling G-protein

transducin in PDE6. The amino terminal region also contains protein- and membrane-targeting sequences in several PDE3:s and PDE4:s, as well as protein kinase phosphorylation sites in PDE:s 1, 3, 4 and 5. These phosphorylation sites are likely to be important in regulation of catalytic activity and/or subcellular location (Perry and Higgs, 1998).

Amongst the cAMP degrading phosphodiesterases, we focus here on the largest and most diverse family known, the PDE4:s. PDE4 enzymes share a common structure, as deduced from their amino acid sequences (Beavo and Reifsnyder, 1990; Bolger *et al.*, 1993, Houslay, Sullivan and Bolger, 1998). Members of each gene family (PDE4A, PDE4B, PDE4C, PDE4D) share common C-terminal regions, different for each family, and catalytic domains that for all PDE4 isoforms are very similar (84% homology over about 360 amino acids across all PDE4:s; Houslay, Sullivan and Bolger, 1998). From N-terminus to catalytic region, the sequence in "long form" PDE4s can be divided into 5 regions, three of which are isoform-specific (N-terminal region, linker regions 1 and 2, or LR1 and LR2) and two, more conserved regions, that are broadly similar between all isoforms, the upstream conserved regions 1 and 2 (UCR1 and UCR2). "Short form" PDE4:s, e.g. PDE4A1, PDE4B2, PDE4D1, PDE4D2, lack UCR1 and LR1 plus differing amounts of the N-terminal region of UCR2. Throughout all regions are potential phosphorylation sites for a variety of kinases, including PKA (e.g. Ser 54 in human PDE4D3), mitogen activated protein kinases (e.g. Ser 487 of human PDE4B2), casein kinase II (e.g. Ser 489 of PDE4B2) and calcium-diacylglycerol dependent protein kinases (Houslay, Sullivan and Bolger, 1998). Phosphorylations at some of these sites have been shown to activate the PDEs (e.g. Ser 54), others serve to inhibit. There is also evidence that some phosphorylations serve to prime the enzymes ready for subsequent activation by further phosphorylation at a different site or sites (Houslay, Sullivan and Bolger, 1998). Other auto-regulatory sites may be found in the N-terminal sequence of certain PDE4:s (Bolger *et al.*, 1996, McPhee *et al.*, 1995).
The identification of rolipram (Schering AG, Berlin, Germany) as an effective inhibitor of PDE4:s (Wachtel, 1982, Nemoz *et al.*, 1985) gave an important tool by which to determine the role of PDE4:s in different cell types. Originally developed as a neurotropic agent, rolipram indicated the therapeutic potential of PDE4 inhibition in control of depressive disorders. Analysis of the pharmacological properties of rolipram, and over 800 publications covering these properties have appeared over the period 1993 to 1998 alone, now indicates that specific PDE4 inhibition may be useful over a very wide range of disease areas. These include: asthma, atopic dermatitis, depression, reperfusion

injury, septic shock, toxic shock, autoimmune diabetes, AIDS, Crohn's disease, multiple sclerosis, cerebral ischemia, psoriasis, allograft rejection, restenosis, ulcerative colitis, cachexia, cerebral malaria, allergic rhinoconjunctivitis, osteoarthritis, rheutmatoid arthritis, autoimmune encephalomyelitis (Houslay, Sullivan and Bolger, 1998).

In the area of asthma, PDE4 inhibition helps to increase cAMP in bronchial smooth muscle, thereby producing a modest bronchodilatory effect, of use in the alleviation of asthmatic symptoms. But perhaps most importantly, inhibition of PDE4:s is now a recognised method by which to suppress immune and inflammatory cell responses (Hughes *et al.*, 1997; Torphy, 1998; Teixeira *et al.*, 1997).

PDE4:s play major roles in modulating the activity of virtually every cell type involved in the inflammatory process. Immune and inflammatory conditions occur when recruitment of leukocytes from the blood compartment into tissues is either uncontrolled, inappropriate, prolonged or directed against self. In asthma, rheumatoid arthritis and multiple sclerosis, infiltration of tissues with inflammatory cells is prolonged and intense, leading ultimately to severe (and self-perpetuating) damage and loss of function. Acute disregulation of the immune system occurs in such conditions as acute respiratory distress syndrome (ARDS) where an overwhelming and generalised inflammatory response can frequently lead to death. There is also substantial evidence which suggests that inflammation may play a part in defining the extent of injury resulting from reperfusion following ischaemia, at least in brain and lung (Entman and Smith, 1994). Chronic inflammatory conditions such as asthma are currently treatable with steroids, but long term treatment brings unavoidable side-effects including immunosuppression, metabolic disturbance and hypertension (Teixeira *et al.*, 1997). Symptoms of rheumatoid arthritis can be alleviated by non-steroidal anti-inflammatories (NSAIDS), but again their side effects are of great concern. Acute conditions such as ARDS have no current treatment as such, only supportive care. Effective anti-inflammatories able to control disregulated reponses, but without the side effects associated with NSAIDS and steroids, have not yet been found.

Within the context of asthma, elevation of intracellular cAMP by PDE inhibition has been associated with inhibition of the function of various types of cells involved in the inflammatory response, including lymphocytes, monocytes, macrophages, neutrophils, eosinophils, mast cells, basophils, endothelial cells and lung epithelial cells (Nicholson and Shahid, 1994); PDE4:s appear to play the dominant role in neutrophils, basophils, eosinophils and mast cells, PDE3s being dominant in monocytes/macrophages and lymphocytes. Inhibitors of PDE3s and PDE4:s often interact synergistically in control of

inflammatory response in asthma models (Teixeira *et al.*, 1997). Other PDE:s may be important in inflammatory cells, but their involvement has yet to be clarified or demonstrated.

Increased cAMP modulates myosin light chain kinase (MLCK) activity causing relaxation, and this is the primary effect in bronchial smooth muscle. Useful compounds will relax bronchial smooth muscle slowly and maintain relaxation for sustained periods, but also help reduce inflammatory immune responses to allergens. Although a combined inhibition of PDE3 and PDE4 isozymes seems to relax bronchial smooth muscle most effectively (Raeburn & Advenier,1995) in humans, the possibility of cardiovascular complications is increased by the use of PDE3 inhibitors, and in fact PDE4 inhibitors such as rolipram, alone or in combination with agonists of the β2 adrenoceptors such as salbutamol, are effective bronchorelaxants.

Possible mechanisms (Teixeira *et al.*, 1997) involved in the anti-inflammatory benefits of PDE4 inhibition *in vivo* include:

- Inhibition of the production and release of inflammatory mediators/cytokines.
- Inhibition of leukocyte migration.
- Induction of cytokines with suppressive activity.
- Inhibition of leukocyte activation (degranulation, respiratory burst).
- Inhibition of the expression/upregulation of cell adhesion molecules.
- Induction of apoptosis amongst inflammatory cells.
- Also, stimulation of endogenous steroid and catecholamine release (Pettipher *et al.*, 1996).

Perhaps the most important consequence *in vivo* of selective PDE4 inhibition may be to inhibit chemokine production, especially those that are chemoattractants of leukocytes (Teixeira *et al.*, 1997). Inhibitors of PDE4 are effective suppressers of cytokine production *in vitro* and reduce serum levels of tumor necrosis factor alpha (TNF-α) in animal models of septic shock (Sekut *et al.*, 1995; Pettipher *et al.*, 1996; Prabhakar *et al.*, 1994). Inhibition of TNF-α production may be central to the beneficial effects of PDE4 inhibition in treatment of inflammatory conditions, but inhibition of the release of chemoattractants such as the α-chemokine interleukin-8 and the lipid leukotriene $(LT)B_4$ may also be important for reducing leukocyte recruitment to sites of inflammation (Turner *et al.*, 1994; Griswold *et al.*, 1993).

It is also known however that there are protective effects of PDE4 inhibition which are quite separate from inhibition of release and action of TNF-α and other pro-inflammatory mediators. At higher concentrations than are necessary to inhibit TNF-α release,

rolipram appears to have a direct effect on eosinophils (Teixeira *et al.*, 1994) and eosinophilia. PDE4 inhibition also stimulates macrophages to produce and release the antiinflammatory cytokine interleukin 10 (IL-10) when challenged with lipopolysaccharide (LPS) *in vitro* (Kambayashi *et al.*, 1995; Jilg *et al.*, 1996), and this same effect may be involved in the protective action of methylxanthines, which are general PDE inhibitors, in a murine model of septic shock (Jilg *et al.*, 1996).

Inhibition of neutrophil activation *in vivo* may also be how PDE4 inhibition protects against acute lung injury induced by LPS followed by zymosan in a murine model (Miotla *et al.*, 1995), and in animal models of asthma, it is likely that PDE4 inhibition suppresses allergic inflammation by inhibition of eosinophil activation together with inhibition of mast cell de-granulation (Hughes *et al.*, 1996).

PDE4 inhibition has also been shown to affect the *in vitro* expression and presentation of cell adhesion molecules such as E-selectin by endothelial cells of the microvasculature (Blease *et al.*, 1998; Mòrandini *et al.*, 1996) and increased cAMP also prevents mediator-induced upregulation of β2 integrins on the surface of eosinophils and neutrophils (Teixeira *et al.*, 1996). Inhibition of the cell adhesion components responsible for recruitment of leukocytes and for initiation of tissue infiltration by the inflammatory cells is an important aspect of therapeutic control for inflammatory conditions.

cAMP-elevating agents also enhance apoptotic clearance of various leukocytes *in vitro* (Hallsworth *et al.*, 1996), and this too may be useful effect in the control of inflammation through PDE4 inhibition.

The major cGMP-degrading PDEs are types 1,2,5, 6, 9 and 10 but here we focus on PDE5, since this is the principal cGMP-specific PDE found in airway and vascular smooth muscle, and it is one of the better documented families ofcGMP-specific PDEs. Little is known yet concerning the role of the newly discovered PDE9 and PDE10 isoforms (Soderling *et al.*, 1998; Fisher *et al.*, 1998; Soderling *et al.*, 1999; Fujishige *et al.*, 1999), and the situation is similar for PDE2s, since good inhibitors are as yet unknown for these (Perry and Higgs, 1998). PDE5 is activated by cAK and (10-fold faster) by cGK (Thomas *et al.*, 1990). Phosphorylation of PDE5 is enhanced in the presence of cGMP, and apparently increases the enzyme's V_{max} by 10-fold (Burns *et al.*, 1992). Coupled with PDE3, these interactions form a feedback system to limit cGMP signaling: increased cGMP will increase cAMP through inhibition of PDE3, high cAMP will activate cAK which, in the presence of elevated cGMP will activate PDE5 and therefore stimulate cGMP breakdown. cAMP levels return to baseline as cGMP falls, by re-activation of PDE3. Recent evidence (Pyne *et al.*, 1996; Lochhead *et al.*, 1997)

suggests that PDE5 may have additional protein components associated with it analagous to the gamma subunits of PDE6. The PDE6γ subunits serve to link activation of the G-protein transducin to activation of the PDE. They are subsequently involved in turning off the signal by helping to activate the transducin GTPase. In the case of PDE5, these associated proteins (14 to 18 kDa) may serve to block activation of the enzyme by cGK and cAK, and the blocking ability of these polypeptides appears to be controlled by a G-protein regulated kinase (Pyne *et al.*, 1996).

cGMP-degrading PDEs work in concert with the action of guanylate cyclases, just as cAMP PDE:s and adenylate cyclases together control cAMP levels in cells. Two groups of GCs are known in mammals, the soluble ones and those that are membrane located. GCs from both groups are central to systemic control of blood pressure. Soluble GCs are expressed in almost all cell types of the cardiovascular system including cardiomyocytes, vascular smooth muscle cells (VSMCs), endothelial cells and platelets (Drewett and Garbers, 1994). Soluble GCs contain a prosthetic heme group which binds NO (and CO) and leads to activation of the enzyme: the vasoactive properties of NO are mediated through the cGMP pathway in this way. The membrane located GCs act as receptors for various ligands (among them, natriuretic peptides and guanylin). cGMP-mediated functions of the natriuretic hormone receptors include vascular smooth muscle relaxation as well as regulation of blood volume (Benner *et al.*, 1990).

cGMP interacts with a number of different effector proteins:

a) with certain ion channels e.g. in photoreceptors and olfactory cells, also in heart and kidney (Lincoln & Cornwell, 1993; Biel *et al.*, 1994; Light *et al.*, 1990);

b) with cGMP-dependent protein kinases (cGKI and cGKII), of which "cytosolic" cGKI predominates in the cardiovascular system and has at least 2 splice variants, α and β. cGKIα has 10-fold higher affinity for cGMP than the β variant. Both cGKI variants are found in vascular smooth muscle (Keilbach *et al.*, 1992, Hofmann *et al.*, 1992);

c) at high concentrations, with cAMP-dependent protein kinases (cAK), which being similar to the cGKs have a certain affinity for cGMP, just as the reverse is also true (Vaandrager & de Jonge, 1996). The functional significance of this potential cross-talk between pathways is not yet fully known, but may be connected with the anti-proliferative effects of cGMP (Lincoln *et al.*, 1994);

d) with cGMP-modulated PDEs: cGMP binds to a non-catalytic site of PDE2 and lowers its K_m for cAMP, lowering the baseline level of cAMP achievable by the enzyme. PDE3 catalysis of cAMP is effectively inhibited by cGMP (Pyne *et al.*, 1987), thus in cells where PDE3 predominates, increased cGMP leads to increased cAMP.

Smooth muscle contracts following Ca^{2+}-calmodulin activation of myosin light chain kinase (MLCK). cGK1 relaxes smooth muscle by lowering free cytoplasmic Ca^{2+} levels, but the principal means by which this is accomplished varies considerably between types of smooth muscle, animal species, and the nature of the contractile stimulus being antagonised (Vaandrager & de Jonge, 1996). cGKI has been implicated in: inhibition of G-protein activation of phospholipase C β; activation of Ca^{2+}-ATPase activity at plasma membrane and sarcoplasmic reticulum (SR); hyperpolarisation of membrane potential through activation of Ca^{2+}-activated K^+ channels; inhibition of voltage operated Ca^{2+} channels; stimulation of the Na^+/Ca^{2+} exchanger; inhibition of SR IP_3 receptors. All of these actions require that the normally cytoplasmic cGKs must find membrane located targets, and specific anchor proteins may be involved. cGKI is already known to be targeted to specific anchor proteins of the cytoskeleton (MacMillan-Crow & Lincoln, 1994), and the discovery of further interactions is likely.

Blood pressure elevation to a degree that requires medical treatment is often encountered in up to 15% of an adult population. In only 10-15% of these, a definite cause for the hypertension can be found and in the rest, the "essential hypertension" has to be treated without a hope for cure of the underlying disease. Long-standing elevation of blood pressure, even quite moderate, damages vessels in the heart, kidneys and brain and dramatically increases the risk for coronary heart disease, renal failure and stroke. It has been shown that effective pharmacologic treatment of hypertension substantially reduces morbidity and mortality from these conditions. The finding that endothelial cells produce a local vascular relaxation factor, identified as nitric oxide (NO), that activates guanylyl cyclase and increases cGMP that in turn leads to reduction in vascular smooth muscle cell tone, has opened new possibilities for blood pressure regulation / vasorelaxation based on modulation of the cellular levels of cGMP. A number of the components in the cGMP system displays tissue specific distribution (Vaandrager & de Jonge, 1996; Pyne *et al.*, 1996). This increases the likelihood for improved pharmacological specificity and fewer side-effects when using these as targets for antihypertensive treatment instead of the traditional ones. It is the cGMP-dependent protein kinase (PKG) (Vaandrager & de Jonge, 1996) that is thought to mediate the intracellular effects of cGMP. The cGMP -dependent and -specific phosphodiesterases can serve as connectors to the cAMP system and terminators of cGMP effects (Pyne *et al.*, 1996).

PDE5 has attracted attention since it is selective for degradation of cGMP versus cAMP. Isoform-specific inhibitors for PDE5 are being developed by several companies and one

compound from Pfizer, Sildenafil, has proven selectivity for PDE5 and is currently being marketed as treatment against impotence (Viagra), originally a side-effect resulting from vasorelaxation in the corpus cavernosum. However the screening procedures currently used search only for direct enzymatic inhibitors of PDE and the compounds found are often not selective, inhibiting for instance both PDE 1 and 5 (e.g. Zaprinast (M&B 22948 RPR), Sch 59498 and Sch 51866). By the methods described herein and within appendix A, new chemical entities can be found which primarily will be specific modulators of PDE action, not inhibitors of the enzymatic action *per se*. Preferred compounds will inhibit the site-specific anchoring of PDEs which hydrolyse cGMP, and thereby reduce their effectiveness in controlling local concentrations cGMP within living cells.

The therapeutic potential of selective modulators of cGMP-related PDE action is not restricted to relaxation of smooth muscle cells but also encompasses other effects ascribed to PKG, such as inhibition of platelet activation (Chiu *et al.*, 1997: Vemulapalli *et al.*, 1996), inhibition of endothelial permeability increases in response to vasoactive substances (Raeburn & Karlsson, 1993), inhibition of the differentiation of osteoclasts (Holliday *et al.*, 1997) and light-induced resetting of circadian rythms (Mathur *et al.*, 1996; Liu *et al.*, 1997).

The search for chemical inhibitors of the catalytic activity of specific PDE:s is currently one of the most intensive areas of pharmaceutical research, particularly so for PDE:s 4 and 5. Much progress has been made in this area, with several compounds known to have selective activity for particular families of PDE:s (reviewed in Perry and Higgs, 1998; Hughes *et al.*, 1997; Teixeira *et al.*, 1997). However, there has not yet been found a class of compounds able to select between isoenzymes within the same family, which is where the greatest opportunities lie. Without isoform specificity, certain difficulties can be expected with the use of enzymic inhibitors of PDE:s. Some of these difficulties are outlined below.

In general, the effects a known inhibitor of the catalytic activity of a particular class of PDE:s may have on cyclic nucleotide levels often varies between different cell types. The reasons for this are several, but include: differences in the basal level of cyclase activity in distinct cell types, crosstalk between cAMP and cGMP systems, and differences in local concentrations of substrate within a cell which influences the degree of inhibition that can be attained by a simple competitive enzyme inhibitor (Perry and Higgs, 1998).

First, PDE inhibition is only useful if it produces the appropriate change in the activity of the dependent effectors, for instance activation of cAK when the concentration of cAMP can be increased above a threshold level. The rate of change in concentration depends in part on the activity of the cyclases which generate the cyclic nucleotides, and that basal level of activity differs from isoform to isoform, and therefore from cell type to cell type. In adipocytes, for example, AC activity is high and cAMP levels are kept at baseline only by a correspondingly high PDE activity. Hepatocytes on the other hand have a rather low AC activity. If both cell types share PDE:s of the same family, and are treated with a chemical inhibitor targeting that family, there will be a rapid increase in cAMP within adipocytes and activation of their cAKs, but no activation in hepatocytes, unless the AC is also stimulated.

Second, general inhibition of a particular isoform of PDE can have certain unavoidable consequences on other cyclic nucleotide pathways since cAMP and cGMP systems are often closely interlinked. Much of this crosstalk arises from PDE regulation by cyclic nucleotides. When cGMP increases in platelets (e.g. following nitric oxide stimulation of soluble GC, or PDE5 inhibition) it inhibits PDE3 and causes a concomitant rise in cAMP (Ashida and Sakuma, 1992). In adrenal glomerulosa cells, atrial natriuretic factor elevates cGMP but inhibits cAMP-stimulated aldosterone synthesis via cGMP-stimulation of PDE2 (MacFarland *et al.*, 1991).

Third, the expected effects of PDE inhibition may be modified by differences in local concentrations of substrates, the reason being that most chemical inhibitors of PDE action are competitive with substrate, so their therapeutic profile is dependent on both the Michaelis-Menton equilibrium constant (K_M) and the substrate concentration in which they are operating (Perry and Higgs, 1998). Most effective inhibition will always occur at lowest substrate levels, but as a corollary, a locally increased substrate level will reduce the inhibition attained. In combination with subtle differences in isoform K_M values for an inhibitor, the desired spatial modulation of cyclic nucleotide levels within a cell could be difficult to obtain by simple competitive inhibition of catalytic activity.

Fourth, there is increasing evidence that cells respond to the prolonged use of agents that increase cyclic nucleotide concentrations by increasing the activity of endogenous levels of appropriate phophodiesterases (Torphy *et al.* 1995), and that one class of mechanism whereby this occurs is by increasing expression levels of PDE proteins (Swinnen *et al.*, 1989, 1991). There is even evidence to suggest that the use of selective inhibitors of different PDE families (eg rolipram for PDE4:s, cilostimide for PDE3, zaprinast for PDE5 etc.), encourages cells and tissues to respond to catalytic inhibition

by upregulating PDE:s specifically of the family type that is under inhibition. Full catalytic inhibition of PDE:s may therefore have self-defeating results, as cells attempt to compensate for lack of specific PDE activity. Careful modulation of local cyclic nucleotide levels within a cell through dislocation or inhibition of redistribution, which may not greatly affect global levels of cyclic nucleotide, may therefore prove to be a better and more effective means to achieve long term therapy.

The radically different methods of interference with PDE action as proposed below in this application should avoid many of the problems outlined above, principally because interference will be family and isoform specific and targeted not against catalytic activity of the PDE:s, but their spatial organisation within the cell.

Targeting of signalling enzymes is a recognised mechanism by which sensitivity, specificity, precision and control may be introduced into intracellular signalling pathways (Pawson and Scott, 1997; Faux and Scott, 1996). The importance and occurrence of targeting as a phenomenon are described and discussed in appendix A. Of central importance to this application is the modulation of the effectiveness of signalling PDE:s through interference with their intracellular targeting. As already described, the many PDE:s known share much structural homology, and this is especially true within the catalytic regions found towards the carboxylic acid terminals of the proteins. At the amino terminals much more heterogeneity is found, between families of PDE:s, between isoforms within families, and between splice variants derived from individual gene isoforms (Houslay and Milligan, 1997). Much of this heterogeneity appears to be associated with differences in targeting behaviour, at least in PDE4 isoforms and variants (Scotland *et al.*, 1998, Bolger *et al.*, 1997), and by extension should apply to other PDEs as well since they are in overall character similar protein molecules with similar roles in cellular signalling.
Evidence suggests that the amino terminal regions of PDE:s can serve to target isoforms to specific intracellular sites (Shakur *et al.*, 1995; McPhee *et al.*, 1995; Bolger *et al.*, 1996; Pooley *et al.*, 1997) and that they can regulate the functioning of the catalytic unit either through interaction with binding proteins (Shakur *et al.*, 1995; O'Connell *et al.*, 1996; Pyne *et al.*, 1996) or through phosphorylation (Sette and Conti, 1996). Targeting appears to occur through protein-protein interactions with membrane- or cytoskeletally-located proteins (Houslay, Sullivan and Bolger, 1998), and of these the membrane associated proteins include both integral and peripherally adherent species. Such

interactions have been probed at a gross level through the use of nonionic detergents and elevated ionic strength (Scotland *et al.*, 1998).

Four separate genes are known to produce PDE4:s in human and rat (PDE4A-D), and each of these produces multiple splice variants (more than 20 described to June 98), many with unique amino terminal regions (Huston *et al.*, 1997; Bolger *et al.*, 1997; Obernolte *et al.*, 1997). Some variants have extensive deletions, even to the point of removing catalytic activity (Obernolte *et al.*, 1997). Differences in the amino terminal regions are presently contemplated to be important for determining differences in the subcellular localisation, activity and sensitivity to inhibitors amongst PDE4 isozymes (Bolger, 1997; Scotland *et al.*, 1998). As an example, PDE4D1 and PDE4D2 are found only in cytosolic fractions, PDE4D3, D4 & D5 are all represented in both cytosolic and particulate fractions. PDE4D3 and D5 are both more sensitive to rolipram inhibition in the cytosolic phase than they are in the particulate fraction (Bolger *et al.*, 1997). Of the 3 "B" isozymes, PDE4B2 is approximately 10 fold more sensitive to rolipram in the particulate fraction than in the cytosolic (Huston *et al.*, 1997). Certain PDE4 isozymes are known to have restricted tissue distributions, e.g. PDE4A8 and PDE4C-delta54 are found only in testis, PDE4C-791 in lung and a melanoma cell line G361 (Bolger *et al.*, 1996; Obernolte *et al.*, 1997). In other cells the expression of isozymes changes with cellular differentiation (Verghese *et al.*, 1995; Giorgi *et al.*, 1997; Bolger *et al.*, 1994; Essayan *et al.*, 1997).

Certain PDE4 isozymes are known to associate with membranes, some with proteins bearing SH3 domains, and some to be purely cytosolic (Scotland *et al.*, 1998; Bolger *et al.*, 1997). A variant of PDE4A ("RD1") transfected into human thyroid carcinoma lines accumulates specifically in Golgi, and at the same time inhibits all expression of "native" PDE1 in those cells (Pooley *et al.*, 1997). These distinct locations are believed to reflect very different functions of the specific phosphodiesterases. A very clear demonstration of functional separation of PDE:s has been seen in renal mesangial cells. Immuno-inflammatory stimulation of these cells increases their production of reactive oxygen metabolites (ROM) and simultaneously increases proliferation. Specific inhibition of PDE4 suppresses ROM production, but not proliferation. Specific inhibition of PDE3 inhibits proliferation but not ROM production (Chini *et al.*, 1997). Both responses are mediated by PKA but control of the cAMP pool is effectively separated.

Location of PDE:s to membranes brings them into contact with phospholipids. Certain PDE4 isozymes are activated by anionic phospholipids such as phosphatidyl serine and

phosphatidic acid (Disanto *et al.*, 1995; Nemoz *et al.*, 1997). Dislocation from the membrane will inhibit such activation, and crosstalk with phopholipid signalling systems. Targeting or anchoring of PDE4:s is likely to have its greatest effect through compartmentalisation of cAMP signalling within cells (Houslay and Milligan, 1997). Associated with the PDE4:s will be specific ACs together with specific isoforms of the effector cAK, or cAMP-operated ion channels. cAKs will likely be attached to specific AKAPs (A-kinase anchoring proteins). Specific subcellular distributions of these components have been mapped in cells (Houslay and Milligan, 1997; Scott and Pawson, 1997; Coghlan *et al.*, 1995) and allow for spatial and temporal gradients of cAMP to be established within cellular compartments. Targeted PDE4 species might serve to control threshold levels of cAMP in the environs of specific cAK molecules, perhaps protecting certain protein complexes from cAK-mediated phosphorylation or manipulating the activity levels of ACs that are necessary before cAK activation may occur.

Competitive chemical inhibitors are known which can selectively inhibit members of the PDE4 family. There are none known which can effectively select between the different gene products or splice variants of the PDE4 family (Perry and Higgs, 1998). This may be due to the particularly high degree of sequence homology within the proteins of this family around the catalytic site. Without splice-variant selectivity, there are likely to be problems with long-term administration of PDE4 inhibitors, such as immunosuppression and metabolic disturbances, possibly with significant CNS effect as well (Teixeira *et al.*, 1997) since PDE4:s are clearly involved in such a wide range of systems at the organismal level. For the family of PDE4 enzymes, the pyrollidone compound rolipram remains the "gold standard" reference inhibitor. However, its profile of serious side effects prevented rolipram from becoming a compound of clinical utility. Principal side effects of rolipram are headaches, nausea, emesis and an unacceptable increase in gastric acid secretion (Barnes, 1995). The PDE4 family is likely to consist of more than the 20 or so isoforms already known in humans (Houslay, Sullivan and Milligan, 1998). Although a potent inhibitor of all known isoforms of PDE4s, the kinetics of inhibition are complex and sensitivity varies significantly from isoform to isoform, and even for individual isoforms in different cell backgrounds or cellular compartments (Bolger *et al.*, 1996; Huston *et al.*, 1996; Jacobitz *et al.*, 1996; McPhee *et al.*, 1995; Owens *et al.*, 1997; Wilson *et al.*, 1994). The side effects of rolipram clearly indicate the potential problems associated with general PDE4 inhibition, while different isoform sensitivities, and changing sensitivities in different cellular contexts, highlights the potential functional

diversity of the many PDE4 isoforms known, and therfore the therapeutic potential that lies in selective inhibition of individual isoforms.

So far only two PDE5 genes are known and two enzyme variants have been reported. In parallel with other PDE isoforms more splicing variants are to be expected from each gene. The enzyme is a homodimer, each subunit being 93 kDa. The structural organisation of the dimer is very similar to that of the cGKs.
PDE5s exist in two distinct forms: one membrane-bound (mPDE5) and one cytosolic (cPDE5) (Pyne *et al.*, 1996). The mPDE5 is activated by PKA and is inhibited by a G-protein dependent mechanism. It is assumed that cPDE5 is part of a "signalling cassette" with NO-regulated guanylate cyclase and PDE3. The latter construction will lead to very short-lived messages whereas the former allows for generation of prologed cGMP signals
Targeting or anchoring of PDE5s is likely to have its greatest effect through compartmentalisation of cGMP signalling within cells. Associated with the PDE5s will be specific GCs together with specific isoforms of the effector cGK, or cGMP-operated ion channels. cGKs may be attached to specific G-kinase anchoring proteins. Specific subcellular distributions of these components will allow for spatial and temporal gradients of cGMP to be established within cellular compartments. Targeted PDE5 species might serve to control threshold levels of cGMP in the environs of specific cGK molecules, perhaps protecting certain protein complexes from cGK-mediated phosphorylation or manipulating the activity levels of GCs that are necessary before cGK activation may occur.
Competitive chemical inhibitors are known which can selectively inhibit PDE5s. Relatively few isoforms of PDE5 are known to date. PDE5 is found rather specifically in vascular and airway smooth muscle. That sildenafil, with its 5 nM IC_{50} for PDE5, affects only a subset of vascular smooth muscle is puzzling, but strongly suggests that either multiple PDE5 isoforms or states exist in different vascular smooth muscle, presumably with different sensitivities to sildenafil, or more likely, other cGMP-hydrolysing PDEs are important in different vascular smooth muscles.
As to other potentially important cGMP-hydrolysing PDE targets, many are doubtless yet to be discovered. PDE9:s have only been known since the end of 1997, PDE10:s since late 1998. PDE9:s have a rather general distribution (kidney, brain, lung), have a very high affinity for cGMP (about 70 nM) and are inhibitable by the PDE1/5 inhibitor SCH51866 (1.55 µM), but "not by sildenafil" (7 µM, Soderling *et al.*, 1998). Their

physiological roles and regulation have not been defined (Soderling *et al.*, 1998; Fisher *et al.*, 1998), but the best suggestions are that they may be involved in keeping cGMP at very low levels when activated, and may, in kidney, be involved in termination of ANP signalling, and therefore inhibition may help potentiate natriuresis without causing deleterious drops in blood pressure (Soderling *et al.*, 1998).

It is clear that PDEs possess heterogenity, particularly in their amino terminal, or "regulatory" regions, and the approach outlined in this application exploits those differences between isoforms and splice variants to produce what should be confined and defined therapeutic effects. Furthermore, in many cases it may be expected that dislocation of an active enzyme from a targeted site of action will have little effect on average cellular concentrations of their preferred cyclic nucleotide substrate, although significant increases may occur at the now PDE-free site of action. This may have significance where an acute short-term process is the therapeutic target, but an integrative gene-regulation effect may occur upon general, non-specific PDE inhibition and overall cyclic nucleotide increase in the cell.

Detailed disclosure

In the present specification and claims, the term "influence" covers any influence to which the cellular response comprises a redistribution. Thus, e.g., heating, cooling, high pressure, low pressure, humidifying, or drying are influences on the cellular response on which the resulting redistribution can be quantified, but perhaps the most important influence is the influence of contacting or incubating the cell or cells with a substance which is known or suspected to cause a redistribution or modify a change of redistribution. In another embodiment of the invention the influence could be substances from a compound drug library.

In the present context, the term "green fluorescent protein" (GFP) is intended to indicate a protein which, when expressed by a cell, emits fluorescence upon exposure to light of the correct excitation wavelength (cf. Chalfie, M. *et al.* (1994) Science 263, 802-805). In the following, GFP in which one or more amino acids have been substituted, inserted or deleted is also termed "modified GFP". "GFP" as used herein includes wild-type GFP derived from the jelly fish *Aequorea victoria* and modifications of GFP, such as the blue fluorescent variant of GFP disclosed by Heim et al. (Heim, R. *et al.* (1994).

Proc.Natl.Acad.Sci. 91:26, pp 12501-12504), and other modifications that change the spectral properties of the GFP fluorescence, or modifications that exhibit increased fluorescence when expressed in cells at a temperature above about 30°C described in PCT/DK96/00051, published as WO 97/11094 on 27 March 1997 and hereby incorporated by reference, and which comprises a fluorescent protein derived from *Aequorea* Green Fluorescent Protein or any functional analogue thereof, wherein the amino acid in position 1 upstream from the chromophore has been mutated to provide an increase of fluorescence intensity when the fluorescent protein of the invention is expressed in cells. Preferred GFP variants are F64L-GFP, F64L-Y66H-GFP and F64L-S65T-GFP. An especially preferred variant of GFP for use in all the aspects of this invention is EGFP (DNA encoding EGFP which is a F64L-S65T variant with codons optimized for expression in mammalian cells is available from Clontech, Palo Alto, plasmids containing the EGFP DNA sequence, cf. GenBank Acc. Nos. U55762, U55763).

The terms "intracellular signalling pathway" and "signal transduction pathway" are intended to indicate the coordinated intracellular processes whereby a living cell transduces an external or internal signal into cellular responses. Said signal transduction will involve an enzymatic reaction said enzymes include but are not limited to protein kinases, GTPases, ATPases, protein phosphatases, phospholipases and cyclic nucleotide phosphodiesterases. The cellular responses include but are not limited to gene transcription, secretion, proliferation, mechanical activity, metabolic activity, cell death.

The term "second messenger" is used to indicate a low molecular weight component involved in the early events of intracellular signal transduction pathways.

The term "luminophore" is used to indicate a chemical substance which has the property of emitting light either inherently or upon stimulation with chemical or physical means. This includes but is not limited to fluorescence, bioluminescence, phosphorescence, chemiluminescence.

The term "mechanically intact living cell" is used to indicate a cell which is considered living according to standard criteria for that particular type of cell such as maintenance of normal membrane potential, energy metabolism, proliferative capability, and has not

experienced any physically invasive treatment designed to introduce external substances into the cell such as microinjection.

In the present context, the term "permeabilised living cell" is used to indicate cells where a pore forming agent such as Streptolysin O or *Staphylococcus Aureus* α-toxin has been applied and thereby incorporated into the plasma membrane in the cells. This creates proteinaceous pores with a defined pore size in the plasma membranes of the exposed cells. Pores could also be made by electroporation, i.e. exposing the cells to high voltage discharges, a procedure that creates small holes in the plasma membrane by coagulating integral membrane proteins. Treatment with a mild detergent such as saponin may accomplish the same thing. Common to all these treatments is that pores are formed only in the plasma membrane without affecting the integrity of cytoplasmic structural elements and organelles. The term living in this context means that the permeabilised cell or cells bathed in a solution mimicking the intracellular milieu still have functional organelles, such as actively respiring mitochondria and endoplasmatic reticulum that can take up and release calcium ions, and functional structural elements. In one embodiment this method is applied so that substances that normally can not traverse the plasma membrane, but most likely exert their influence intracellularly, can be introduced and their influence studied. In another embodiment this method is used to record the response to an influence from many cells simultaneously.

In the present context, the term "permeabilisation" is intended to indicate the selective disruption of the plasma membrane barrier so that soluble substances freely mobile in the cytosol may be lost from the interior of the cells. The permeabilisation can be achieved as described above under "permeabilised living cells" or by using other chemical detergents such as Triton X-100 or digitonin in carefully titrated amounts.

The term "physiologically relevant", when applied to an experimentally determined redistribution of an intracellular component, as measured by a change in the luminescence properties or distribution, is used to indicate that said redistribution can be explained in terms of the underlying biological phenomenon which gives rise to the redistribution.

The terms "image processing" and "image analysis" are used to describe a large family of digital data analysis techniques or combination of such techniques which reduce

ordered arrays of numbers (images) to quantitative information describing those ordered arrays of numbers. When said ordered arrays of numbers represent measured values from a physical process, the quantitative information derived is therefore a measure of the physical process.

The term "mammalian cell" is intended to indicate any living cell of mammalian origin. The cell may be an established cell line, many of which are available from The American Type Culture Collection (ATCC, Virginia, USA) or a primary cell with a limited life span derived from a mammalian tissue, including tissues derived from a transgenic animal, or a newly established immortal cell line derived from a mammalian tissue including transgenic tissues, or a hybrid cell or cell line derived by fusing different celltypes of mammalian origin e.g. hybridoma cell lines. The cells may optionally express one or more non-native gene products, e.g. receptors, enzymes, enzyme substrates, prior to or in addition to the fluorescent probe. Preferred cell lines include but are not limited to those of fibroblast origin, e.g. BHK, CHO, BALB, or of endothelial origin, e.g. HUVEC, BAE (bovine artery endothelial), CPAE (cow pulmonary artery endothelial), HLMVEC (human lung microvascular endothelial cells), or of airway epithelial origin, e.g. BEAS-2B, or of pancreatic origin, e.g. RIN, INS-1, MIN6, bTC3, aTC6, bTC6, HIT, or of hematopoietic origin, e.g.primary isolated human monocytes, macrophages, neutrophils, basophils, eosinophils and lymphocyte populations, AML-14, AML-193, HL-60, RBL-1, U937, RAW, JAWS, or of adipocyte origin, e.g. 3T3-L1, human pre-adipocytes, or of neuroendocrine origin, e.g. AtT20, PC12, GH3, muscle origin, e.g. SKMC, A10, C2C12, renal origin, e.g. HEK 293, LLC-PK1, or of neuronal origin, e.g. SK-N-DZ, SK-N-BE(2), HCN-1A, NT2/D1.

The term "hybrid polypeptide" is intended to indicate a polypeptide which is a fusion of at least a portion of each of two proteins, in this case at least a portion of the green fluorescent protein, and at least a portion of a catalytic and/or regulatory domain of a protein kinase. Furthermore a hybrid polypeptide is intended to indicate a fusion polypeptide comprising a GFP or at least a portion of the green fluorescent protein that contains a functional fluorophore, and at least a portion of a biologically active polypeptide as defined herein provided that said fusion is not the Glucocorticoid Receptor-GFP disclosed by Carey, KL et al. and Guiliano, KA et al., respectively. Thus, GFP may be N- or C-terminally tagged to a biologically active polypeptide, optionally via a linker portion or linker peptide consisting of a sequence of one or more amino acids.

The hybrid polypeptide or fusion polypeptide may act as a fluorescent probe in mechanically intact or permeabilised living cells carrying a DNA sequence encoding the hybrid polypeptide under conditions permitting expression of said hybrid polypeptide. The term hybrid polypeptide or fusion polypeptide is intended also to include the term "fluorescent probe", where the latter is used to indicate a fluorescent fusion polypeptide comprising a GFP or any functional part thereof which is N- or C-terminally fused to a biologically active polypeptide as defined herein, optionally via a peptide linker consisting of one or more amino acid residues, where the size of the linker peptide in itself is not critical as long as the desired functionality of the fluorescent probe is maintained. A fluorescent probe according to the invention is expressed in a cell and basically mimics the physiological behaviour of the biologically active polypeptide moiety of the fusion polypeptide.

The term "kinase" is intended to indicate an enzyme that is capable of phosphorylating a cellular component.

The term "protein kinase" is intended to indicate an enzyme that is capable of phosphorylating serine and/or threonine and/or tyrosine in peptides and/or proteins.

The term "phosphatase" is intended to indicate an enzyme that is capable of dephosphorylating phosphoserine and/or phosphothreonine and/or phosphotyrosine in peptides and/or proteins.

The term "cyclic nucleotide phosphodiesterase" is intended to indicate an enzyme that is capable of inactivating the second messengers cAMP and cGMP by hydrolysis of their 3'-ester bond.

In the present context, the term "biologically active polypeptide" is intended to indicate a polypeptide affecting intracellular processes upon activation, such as an enzyme which is active in intracellular processes or a portion thereof comprising a desired amino acid sequence which has a biological function or exerts a biological effect in a cellular system. In the polypeptide one or several amino acids may have been deleted, inserted and/or replaced to alter its biological function, e.g. by rendering a catalytic site inactive or by disrupting the targeting sequence. In another embodiment, one or several amino acids may have been deleted, inserted and/or replaced without altering the biological function

of the polypeptide, that is, it remains biologically equivalent. Preferably, the biologically active polypeptide is selected from the group consisting of proteins taking part in an intracellular signalling pathway, such as enzymes involved in the intracellular phosphorylation and dephosphorylation processes including kinases, protein kinases and phosphorylases as defined herein, but also proteins making up the cytoskeleton play important roles in intracellular signal transduction and are therefore included in the meaning of "biologically active polypeptide" herein. More preferably, the biologically active polypeptide is a protein which according to its state as activated or non-activated changes localisation within the cell, preferably as an intermediary component in a signal transduction pathway. Included in this preferred group of biologically active polypeptides are cAMP dependent protein kinases, 'inhibitor of NF-kappaB' kinases, and cyclic nucleotide phosphodiesterases.

The term "a substance" is intended to indicate any sample which has a biological function or exerts a biological effect in a cellular system. The sample may be a sample of a biological material such as a sample of a body fluid including blood, plasma, saliva, milk, urine, or a microbial or plant extract, an environmental sample containing pollutants including heavy metals or toxins, or it may be a sample containing a compound or mixture of compounds prepared by organic synthesis or genetic techniques.

The phrase "any change in fluorescence" means any change in absorption properties, such as wavelength and intensity, or any change in spectral properties of the emitted light, such as a change of wavelength, fluorescence lifetime, intensity or polarisation, or any change in the intracellular localisation of the fluorophore. It may thus be localised to a specific cellular component (e.g. organelle, membrane, cytoskeleton, molecular structure) or it may be evenly distributed throughout the cell or parts of the cell.

The term "organism" as used herein indicates any unicellular or multicellular organism preferably originating from the animal kingdom including protozoans, but also organisms that are members of the plant kingdoms, such as algae, fungi, bryophytes, and vascular plants are included in this definition.

The term "nucleic acid" is intended to indicate any type of poly- or oligonucleic acid sequence, such as a DNA sequence, a cDNA sequence, or an RNA sequence.

The term "biologically equivalent" as it relates to proteins is intended to mean that a first protein is equivalent to a second protein if the cellular functions of the two proteins may substitute for each other, e.g. if the two proteins are closely related isoforms encoded by different genes, if they are splicing variants, or allelic variants derived from the same gene, if they perform identical cellular functions in different cell types, or in different species. The term "biologically equivalent" as it relates to DNA is intended to mean that a first DNA sequence encoding a polypeptide is equivalent to a second DNA sequence encoding a polypeptide if the functional proteins encoded by the two genes are biologically equivalent.

The term "fixed cells" is used to mean cells treated with a cytological fixative such as glutaraldehyde or formaldehyde, treatments which serve to chemically cross-link and stabilize soluble and insoluble proteins within the structure of the cell. Once in this state, such proteins cannot be lost from the structure of the now-dead cell.

In the present context a "quantitative fluorescence redistribution assay" is intended to indicate an assay whereby it is possible to observe and quantify the subcelluar localisation and possible redistribution of an biologically active polypeptide, or part thereof, genetically or chemically tagged with a luminophore inside an intact living cell or cells or permeabilised living cells. The subcelluar location and redistribution may be monitored using fluorescence microscopy or fluorescence imaging microscopy but is preferably monitored using a fluorescence imaging plate reader or a fluorescence plate reader for improved throughput. A more thorough description is given in Appendix A.

In the present context a "mortal cell line" is used to indicate animal cells that may grow in vitro, given the right conditions, but that have a definite life span of a number of cell divisions or days, week or months beyond which it is not at present possible to keep them alive.

In the present context an " immortalised cell line" is used to indicate cells of animal origin where the normal limitations for cell life and number of cell divisions do not apply. Essentially, such cells can live, grow and divide for an unlimited or very long (years to decades) time.

The term "targeting sequence" is used to indicate the amino-acid sequence of a biologically active polypeptide that contains the actual structure or structures necessary for association of the biologically active polypeptide with its native intracellular binding sites. The term "targeting sequence" is also used to indicate the amino-acid sequence of a protein that contains the actual structure or structures necessary for association of a biologically active polypeptide with the protein.

The term "targeting" is used to indicate the process whereby a spatially distributed protein is directed to the intracellular sites and maintained at the intracellular sites to which it is normally anchored or associated. These anchoring sites are normally assumed to be the intracellular sites where the protein has its optimal function for the cell.

The term "dislocate" and derivatives thereof is used to indicate the process whereby an intracellularly spatially distributed protein is forced to detach from its normal anchoring or association structures in the cells due to intercalation of another, preferably smaller, compound at the site of anchoring or association. This usually means that the optimal function of the protein within the cell is lost or reduced and that a larger portion of the protein molecules are freely mobile within the cytoplasm.

In the present context a "screening assay" is intended to mean any measurement protocol, including materials, cells, instruments, chemicals, reagents, detection units, calibration and quantification procedures used to measure a response from mechanically intact or permeabilised living cells relevant to influences on an intracellular pathway.

In the present context a "primary screening assay" is used to indicate the first screening assay in a discovery project that is used to select and sort all compounds available to the project according to the quantified effect of the compounds in the assay.

In the present context a "counterscreen" is intended to mean a screening assay that is relevant to a phenomenon that is undesirable seen from the point of view of the discovery project.

In the present context a "discovery project" is intended to mean the process whereby general or specific ideas about ways of how to modulate an intracellular signalling

pathway are exploited in order to find new chemical compounds that can be used to modulate the intracellular signalling pathway and thereby treat, reduce or abolish symptoms associated with a condition or a disease that is lethal, degenerative, performance-reducing or just uncomfortable to an animal, preferably a human being. The aim of the discovery project is to produce drug candidates that can be tested as potential drugs in an animal, preferably in human beings. The term "discovery project" also encompasses the actual group of individuals, screening assays, tests, machinery, cells, animals and compounds involved in different aspects of the project.

The term "tagging" is used to indicate the process whereby a luminophore is genetically or chemically attached to the protein, or part of the protein, of interest to the discovery project.

The term "primary hit" is used to indicate compounds identified in the primary screening assay as having at least the minimum level of desired effect that has been specified in the discovery project.

The term "primary lead compound" is used to indicate a primary hit that has at least the minimal level of desired potency and specificity predetermined by the discovery project.

The term "dose-response relationship" is in the present context intended to mean a clear correlation between the quantified response of cells in a screening assay to application of an influence, such as a compound, and the concentration of the applied influence. The response to the influence may be both an up-regulation and a down-regulation of the quantitated parameter used in the screening assay.

In the present context, the term "potency" is intended to mean the ability of an influence to affect the process under study. The process under study may be, for example a screening assay or a specific physiological or pathophysiological response in an animal.

In the present context, the term "selectivity" is intended to mean the difference in potency on the desired process, such as a screening assay, and an undesired process, such as a counterscreen, with the view of the discovery project. An influence or a compound is said to display selectivity if the potency for the desired process is higher than for the undesired process.

In the present context, the term "structure-activity relationship" or "SAR" is intended to mean the situation where a direct relationship exists between a compound and modifications made to the compound and the activity of the compound and the modifications made to the compound in one or more screening assays. The process of building a SAR may be used to direct the chemical construction of new compounds with higher potency and selecivity than the original compound.

The term "drug candidate lead" is used to indicate compounds that may be pursued by a discovery project as potential candidates for the final outcome of the project.

In the present context, the term "efficacy" is intended to mean the ability of a compound to affect the process or condition under study. It is closely related to the term "potency" but is in the present context used when relating to effects of a compound on more complex screening assays than the primary screening assay or counterscreens and when relating to effects of a compound in animals.

In the present context, the term "toxicity" is intended to mean that a compound in some way is toxic to cells, tissues or animals. The toxicity means that the cells, tissues or animals will in some way be harmed if the compound is applied at a sufficient concentration. The effects may ultimately lead to cell, tissue or animal death or a limited life compared to the normal condition.

In the present context, the term "physiology" is intended to mean the normal function of biological and biochemical processes inside cells, between cells and in the whole organism or animal.

In the present context, the term "pathophysiology" is intended to mean deviations from the normal function of biological and biochemical processes inside cells, between cells and in the whole organism or animal that may be part of a condition or disease.

In the present context, the term "pathogenesis" is intended to mean the process, be it genetical, biological, biochemical, chemical or environmental, that ultimately may explain, at least in part, the apparent pathophysiology associated with a condition or disease in an animal.

In the present context, the term “fractionated cells” is intended to mean the outcome of a simple division of initially mechanically intact living cells into two fractions, particulate (the components that can be sedimented by centrifugation at more than 10 000xg and not more than 100 000xg for 10 minutes) and soluble fraction (the soluble components and small membrane fragments that do not sediment), after subjecting the cells to plasma membrane disruption either mechanically with some form of homogeniser or sonicator or osmotically (hypoosmotic shock) or through some kind of permeabilisation of the plasma membrane with detergents, toxins or electroporation.

The term “parenteral route of administration” is used to indicate the administration of a drug or compound in solution to an animal, such as a mammal or a human, by injection or infusion of the drug or compound into the bloodstream of the animal via an injection needle iserted into one of the animals blood vessels, preferably a vein.

The term “oral route of administration” is used to indicate the administration of a drug or compound in solution or as a solid to an animal, such as a mammal or a human, by placing the drug or compound in the mouth of the animal so that the animal itself can swallow the drug or compound or have it delivered to the stomach or intestine by intubation. When the drug or compound enters the stomach and intestine it will be taken up over the mucosa into the bloodstream and administered via the blood stream to the tissues and organs where it is to exert its effect, or it will be acting locally in the stomach and intestine.

The term “pulmonary route of administration” is used to indicate the administration of a drug or compound as an aerosol with either solid or liquid particles to an animal, such as a mammal or a human, by placing the drug or compound container close to or in contact with the mouth and/or nose of the animal so that the animal itself can inhale the drug or compound aerosol. When the drug or compound enters the peripheral bronchioloi and alveoli it will be taken up over the alveolar membrane, either into the bloodstream and administered via the blood stream to the tissues and organs where it is to exert its effect or it will act locally in the lungs on lung, vessel and muscle cells as well as any other cell type present th re.

The term "cutaneous route of administration" is used to indicate the administration of a drug or compound in solution or as a solid to an animal, such as a mammal or a human, by placing the drug or compound on the skin of the animal. The drug can then enter the blood vessels under the skin as it is permeaing the skin and thereby be taken up into the bloodstream and administered via the blood stream to the tissues and organs where it is to exert its effect. It may also exert an effect locally on the site of application on the skin.

The term "rectal route of administration" is used to indicate the administration of a drug or compound in solution or as a solid to an animal, such as a mammal or a human, by placing the drug or compound in the rectal cavity of the animal. When the drug or compound enters the rectum and parts of the large intestine it will be taken up over the mucosa into the bloodstream and administered via the blood stream to the tissues and organs where it is to exert its effect, or it will act locally in the rectum and parts of the large intestine.

Several IKKs and very many phosphodiesterases (PDE:s) are known. They are grouped in families according to functional criteria. Within each family there may be several members - isoforms- encoded by different genes. Each isoform may give rise to several splice variants. This hierarchy is evidenced at the sequence level: isoforms are more similar to each other than to members of other families; splice variants are more similar to each other than to other PDE:s. Each specific PDE thus contains sequences that are unique to itself, as well as sequences that are shared between isoforms and/or families. When setting up a program to identify pharmacological agents that affect the intracellular distribution of a target IKK or PDE, it is first necessary to choose the target from the IKKs and PDE:s known. This may be done according to various criteria. A first criterion is that it is imperative that the target IKK or PDE be present in the tissue or cell type(s) where the pharmacological agent is to exert its effect. A second criterion is that it is desirable that either the target or a specific anchoring/targeting site not be present in tissues or cell types where no pharmacological effects are desired.

Establishing the expression patterns of IKKs and PDE:s in relation to tissues and cell types is best done using the methods of detection of mRNA, e.g. Northern analysis, which is a well established procedure. Briefly, mRNA isolated from a given source is probed with a labelled nucleotide, whose sequence is complementary to the mRNA or a region in a mRNA of interest. The assay allows the investigator to determine the

stringency of the probing, i.e. to correlate the resulting signal(s) with sequence similarities.

As a first step, the nucleotide sequences of IKKs or PDE:s are compiled and inspected to identify regions that are unique to specific IKKs or PDE:s as well as regions that are shared among several, many, or all IKKs or PDE:s. Nucleotide sequences may be found in a depository of genetic information, e.g. GenBank, which is a well known resource. The inspection of the sequences may be aided by using computer programs that were developed to align several or many sequences, and in so doing highlighting regions of similarity or lack of the same. Many of these are presented and explained in great detail in e.g. Sequence Data Analysis Guidebook /edited by S.R.Swindell, Methods in Molecular Biology vol. 70 (1997), from Humana Press Inc. Totowa, New Jersey. When sequences have been identified that are unique to an IKK, or a PDE, or respectively shared by several or many IKKs or PDE:s, oligonucleotide probes based on these sequences may be designed and synthesized. The use of such probes to detect mRNA is well established in the research community, see e.g. Basic DNA and RNA Protocols/edited by A.J.Harwood, Methods in Molecular Biology vol. 58 (1996), from Humana Press Inc. Totowa, New Jersey. E.g. Life Technologies offer to synthesize specified oligonucleotides.

In addition to oligonucleotide probes, mRNA extracted from the tissues and cell types of interest is required, preferably in a form ready to use in Northern analysis. Several companies offer such material, e.g. Invitrogen and Clontech. Briefly, they provide RNA extracted from a great many human and non-human tissues or cell types immobilized on membranes, as an array or size-fractionated.

In a next step, a detectable label needs to be attached to the oligonucleotide probe(s). The label is traditionally in the form of a radioactive isotope, but may to advantage be a chemiluminescent reagent or a fluorescent agent. See e.g. DNA Probes by Keller and Manak (1993), from Macmillan Publishers. Several companies offer reagents to label nucleotide probes, e.g. Ambion (Austin, Texas) and Molecular Probes (Eugene, Oregon).

The actual probing procedure involves contacting the immobilized mRNA (s) with the probe(s), washing away unbound probe(s) and detecting the signal(s) from the probe(s) that bound under the conditions tested, a positive signal indicating that the target(s) of the probe(s) was present in the sample(s) subjected to the test. In its simplest form, the test is "one-to-one", i.e. each sample of mRNA is exposed to each probe. However, it may be advantageous to exploit the sequence hierarchy of the IKKs or PDE:s, by first

probing arrays of mRNA from multiple sources with family-specific probes, then examining first positives with isotype-specific probes, and then examining the secondary positives in detail with very specific probes. One could also multiplex the probing by adding different distuingishable fluorescent labels to the probes, thus obtaining information from several probes in one experiment.
The outcome of the analysis is information regarding the expression pattern(s) of IKKs and PDE:s.
Based on their expression pattern(s) specific IKKs and/or PDE:s are then selected for further study, and genetic probes are constructed.

In general, a genetic probe, i.e. a "GeneX"-GFP fusion or a GFP-"GeneX" fusion, is constructed using PCR with "GeneX"-specific primers followed by a cloning step to fuse "GeneX" in frame with GFP. The fusion may contain a short vector derived sequence between "GeneX" and GFP (e.g. part of a multiple cloning site region in the plasmid) resulting in a peptide linker between "GeneX" and GFP in the resulting fusion protein. The fusion may be made using ploymerase chain reaction techniques, which are common laboratory procedures, see e.g. PCR Protocols/edited by B.A.White, Methods in Molecular Biology vol. 15 (1993), from Humana Press Inc. Totowa, New Jersey.

In more detail, the steps involved include:

- Design of gene-specific primers. Inspection of the sequence of the gene allows design of gene-specific primers to be used in a PCR reaction. Typically, the top-strand primer encompasses the ATG start codon of the gene and the following ca. 20 nucleotides, while the bottom-strand primer encompasses the stop codon and the ca. 20 preceding nucleotides, if the gene is to be fused behind GFP, i.e. a GFP-"GeneX" fusion. If the gene is to be fused in front of GFP, i.e. a "GeneX"-GFP fusion, a stop codon must be avoided. Optionally, the full length sequence of GeneX may not be used in the fusion, but merely the part which localizes and redistributes like GeneX in response to a signal.

  In addition to gene-specific sequences, the primers contain at least one recognition sequence for a restriction enzyme, to allow subsequent cloning of the PCR product. The sites are chosen so that they are unique in the PCR product and compatible with sites in the cloning vector. Furthermore, it may be necessary to include an exact number of nucleotides between the restriction enzyme site and the gene-specific sequence in order to establish the correct reading frame of the fusion gene and/or a

translation initiation concensus sequence. Lastly, the primers always contain a few nucleotides in front of the restriction enzyme site to allow efficient digestion with the enzyme.

- Identifying a source of the gene to be amplified. In order for a PCR reaction to produce a product with gene-specific primers, the gene-sequence must initially be present in the reaction, e.g. in the form of cDNA. The results of the extensive expression analysis performed previously will provide clear information regarding what tissue(s) are useful as source material. cDNA libraries from a great variety of tissues or cell types from various species are commercially available, e.g. from Clontech (Palo Alto), Stratagene (La Jolla) and Invitrogen (San Diego). Many genes are also available in cloned form from The American Type Tissue Collection (Virginia).
- Optimizing the PCR reaction. Several factors are known to influence the efficiency and specificity of a PCR reaction, including the annealing temperature of the primers, the concentration of ions, notably Mg^{2+} and K^{+}, present in the reaction, as well as pH of the reaction. If the result of a PCR reaction is deemed unsatisfactory, it might be because the parameters mentioned above are not optimal. Various annealing temperatures should be tested, e.g. in a PCR machine with a built-in temperature gradient, available from e.g. Stratagene (La Jolla), and/or various buffer compositions should be tried, e.g. the OptiPrime buffer system from Stratagene (La Jolla).
- Cloning the PCR product. The vector into which the amplified gene product will be cloned and fused with GFP will already have been taken into consideration when the primers were designed. When choosing a vector, one should at least consider in which cell types the probe subsequently will be expressed, so that the promoter controlling expression of the probe is compatible with the cells. Most expression vectors also contain one or more selective markers, e.g. conferring resistance to a drug, which is a useful feature when one wants to make stable transfectants. The selective marker should also be compatible with the cells to be used.

The actual cloning of the PCR product should present no difficulty for the person skilled in the art as it typically will be a one-step cloning of a fragment digested with two different restriction enzymes into a vector digested with the same two enzymes. If the cloning proves to be problematic, it may be because the restriction enzymes did not work well with the PCR fragment. In this case one could add longer extensions to the end of the primers to overcome a possible difficulty of digestion close to a fragment end, or one could introduce an intermediate cloning step not based on restriction enzyme digestion.

Several companies offer systems for this approach, e.g. Invitrogen (San Diego) and Clontech (Palo Alto).
Once the gene has been cloned and, in the process, fused with the GFP gene, the resulting product, usually a plasmid, should be carefully checked to make sure it is as expected. The most exact test would be to obtain the nucleotide sequence of the fusion-gene.

Once a DNA construct for a probe has been generated, its functionality and usefulness may be tested by subjecting it to the following tests:

- Transfecting it into cells capable of expressing the probe. The fluorescence of the cell is inspected soon after, typically the next day. At this point, two features of cellular fluorescence are noted:
- The intensity should usually be at least as strong as that of unfused GFP in the cells. If it is not, the sequence or quality of the probe-DNA might be faulty, and should be carefully checked.
- The sub-cellular localization is an indication of whether the probe is likely to perform well.

If it localizes as expected for the gene in question, e.g. is excluded from the nucleus, it can immediately go on to a functional test. If the probe is not localized soon after the transfection procedure, it may be because of overexpression at this point in time, as the cell typically will have taken of very many copies of the plasmid, and localization will occur in time, e.g. within a few weeks, as plasmid copy number and expression level decreases. If localization does not occur after prolonged time, it may be because the fusion to GFP has destroyed a localization function, e.g. masked a protein sequence essential for interaction with its normal cellular anchor-protein. In this case the opposite fusion might work, e.g. if GeneX-GFP does not work, GFP-GeneX might, as two different parts of GeneX will be affected by the proximity to GFP. If this does not work, the proximity of GFP at either end might be a problem, and it could be attempted to increase the distance by incorporating a longer linker between GeneX and GFP in the DNA construct.
If there is no prior knowledge of localization, and no localization is observed, it may be because the probe should not be localized at this point, because such is the nature of the protein fused to GFP. It should then be subjected to a functional test.

In a functional test, the cells expressing the probe are treated with at least one compound known to perturb, usually by activating, the signalling pathway on which the probe is expected to report by redistributing itself within the cell.
If the redistribution is as expected, e.g. if prior knowledge tell that it should translocate from location X to location Y, it has passed the first critical test. In this case it can go on to further characterization and quantification of the response.
If it does not perform as expected, it may be because the cell lacks at least one component of the signalling pathway, e.g. a cell surface receptor, or there is species incompatibility, e.g. if the probe is modelled on sequence information of a human geneproduct, and the cell is of hamster origin. In both instances one should identify other cell types for the testing process where these potential problems would not apply.
If there is no prior knowledge about the pattern of redistribution, the analysis of the redistribution will have to be done in greater depth to identify what the essential and indicative features are, and when this is clear, it can go on to further characterization and quantification of the response.
If no feature of redistribution can be identified, the problem might be as mentioned above, and the probe should be retested under more optimal cellular conditions.

Libraries for cloning of cDNA libraries in the present discovery plan are naturally related to the target tissues of the projects. For ultimately finding lead compounds useful in the treatment of asthma the cloning libraries should preferably be obtained from one ore more of the following tissue or cells types: Bronchial smooth muscle, Lung microvascular endothelial cells, eosinophil granulocytes, Th1 or 2 lymphocytes and alveolar macrophages.
For ultimately finding lead compounds useful in the treatment of chronic inflammatory diseases the cloning libraries should preferably be obtained from one ore more of the following tissue or cell types: Th1 or 2 lymphocytes, T-lymphocytes, B-lymphocytes, Monocytes, Eosinophil granulocytes, Neutrophil granulocytes, Basophil granulocytes, Tissue specific macrophages (such as the liver Kupffer cells and skin Langhans cells), microvascular endothelial cells, vascular endothelial cells, antigen presenting cells, joint connective and synovial cells. For ultimately finding lead compounds useful in the treatment of depression the cloning libraries should preferably be obtained from one or more of the various tissue regions of the brain containing noradrenergic neurons. For ultimately finding lead compounds useful in the treatment of jet lag or circadian clock

resetting the cloning libraries should preferably be obtained from one or more of the various tissues of the brain such as the pineal gland, hypothalamus and substantia nigra. For ultimately finding lead compounds useful in the treatment of hyper- and hypotension and erectile dysfunction the cloning libraries should preferably be obtained from one or more of the following tissue or cell types: vascular smooth muscle, vascular smooth muscle from resistance vessels on the arterial side of the vascular system, vascular smooth muscle from capacitance vessels on the venous side of the vascular system, vascular smooth muscle cells from small arteries, arterioles, venules or veins, smooth vascular cells lines such as T/G HA-VSMCA10 and A7r5.

The cells should always be of animal origin, most likely of mammalian origin and preferably of human origin. The cells could be derived from normal tissue or from tissue of an individual animal having a disease or condition of interest for the project. The cells may also be a mortal or immortalised cell line where the initial cell clone has been derived from a tissue or cell type as described above. Depending on the discovery project the cells of interest for screening assays will vary but may be chosen from the above mentioned categories.

Once a genetic construct containing the protein of interest and the luminophore, from here on referred to as "the original fluorescent probe", has been transfected into a relevant cell type, as described above under 'preferred cell types for cloning libraries' the cells are monitored for the appearance of spatially distributed or randomly distributed intracellular fluorescence. Based on prior knowledge regarding the distribution of the actual protein different patterns can be expected. If for example previous studies have found the protein associated only with the particulate fraction of fractionated cells, it can be expected to find a spatial distribution of the original fluorescent probe to the plasma membrane, internal membrane/organelle structures or structural cytoplasmic elements such as microtubules and microfilaments. If on the other hand previous studies report that the protein has been found mostly in the soluble fraction of fractionated cells one can expect to find a homogenous or nonhomogenous distribution of the original fluorescent probe throughout the cytoplasm and perhaps also in the nucleus. For proteins where previous studies have found a mixed localisation to both the particulate and soluble fraction of fractionated cells any mixture in the two distribution patterns mentioned above for the original fluorescent probe can be expected. For proteins where no prior knowledge is at hand a simple cell fractionation and Western Blotting can be

made, one can use immunohistochemistry of fixed cells of relevance or one can decide to rely on the distribution observed for the original fluorescent probe. At this stage of the project, a normal distribution pattern of the original fluorescent probe may be established after such studies as outlined above. The effects of physiologically important and relevant cellular activation on the distributed pattern of the original fluorescent probe is also established. It will also become evident if the pattern of distribution changes, i.e. if a redistribution of the original fluorescent probe occurs as a consequence of applying a physiologically important and relevant influence.

The stategy described herein is used to search for chemical entities which can interfere with the protein-protein interactions that occur amongst biologically active polypeptides and their anchoring/regulating partners, and thereby interfere with the effectiveness of a biologically active polypeptide's action within its cellular environment. The strategy will have different effects, and require slightly different discovery methods depending on the nature of the interaction. The possibililties are as follows:

1) A biologically active polypeptide is permanently located at its targeting point, and either remains permanently active there, or its activity is modulated in some way by post-translational modification such as phosphorylation or by binding of modulators to non-catalytic regulatory sites. Dislocation from the targeting site will remove the biologically active polypeptide from a localised site of action, and may also lead to inactivation of its inherent catalytic activity.

2) A biologically active polypeptide is permanently located at its targeting point, and remains inactive there until its activity is modulated in some way by post-translational modification, such as phosphorylation or by binding of modulators to non-catalytic regulatory sites. Dislocation from the targeting site will remove the biologically active polypeptide from a localised site of action, and may also lead to activation of its inherent catalytic activity, albeit away from its original anchoring site.

3) A biologically active polypeptide is inactive in its unattached or untargeted form, and when activated (as described in "1" above), or partially activated, it redistributes within the cell and becomes attached to its targeting site, its activity being restricted to the anchoring site and possibly enhanced by interaction with the anchoring protein or some associated factor, or at some later time inhibited by the anchoring protein or an associated regulatory factor. Any agent which prevents association of the biologically active polypeptide with its anchoring or targeting site will prevent it from locating to the

preferred site of action, and may also prevent the biologically active polypeptide from becoming fully activated by the appropriate stimulus whilst in the untargeted state.

4) A biologically active polypeptide is active in its unattached or untargeted form, and when inactivated (as described in "1" above), or partially inactivated, it redistributes within the cell and becomes attached to its targeting site, whereby its activity is inhibited by interaction with the anchoring protein or an associated regulatory factor. Subsequent stimuli may then activate and release the biologically active polypeptide. Any agent which prevents association of the biologically active polypeptide with its anchoring or targeting site will prevent it from relocating to the anchoring position, and may also prevent the biologically active polypeptide from ever being inactivated. In addition, if the biologically active polypeptide cannot target to its anchoring site, it may not be possible subsequently to activate the biologically active polypeptide in the appropriate way in the untargeted state.

When a specific subcellular distribution of a GFP-based IKK or PDE probe has been identified, it may be advantageous to narrow down which part of the IKK or PDE is responsible for this effect. The advantage is twofold: It may suggest the design of peptide leads, and it may eventually aid in defining the binding partner. Knowledge of both partners involved in specific binding may aid in the selection of compound libraries to screen for inhibition of the specific binding.

To identify the region of the IKK or PDE involved in specific binding, one may make GFP-based fusions with progressively shorter parts of the IKK or PDE, and examine the cellular distribution of these constructs. If there is prior knowledge of functional domains, one may start with the domain believed to confer specific binding to a subcellular structure. The generation of constructs to test may consist of selecting a particular part of the IKK or PDE to fuse to GFP, or it may involve the generation of in-frame deletions in the IKK or PDE part of the fusion. Both approaches have been widely used in molecular genetic studies.

When a region has been identified that appears responsible for conferring a specific subcellular distribution upon an IKK or a PDE, the amino acid residues most important for this trait may be identified by a more detailed analysis, e.g. substituting them one by one with e.g. an alanine residue, a so called Ala-scan, which also has been used extensively in molecular genetic studies.

To identify the identity of the cellular protein partaking in the specific distribution of the IKK or PDE, one may exploit the knowledge about the region of the IKK or PDE

responsible for the subcellular distribution; for example, one may use the region of the IKK or PDE as bait in a genetic two hybrid screen to pull out its binding partner. Several companies offer two hybrid systems, e.g. Life Technologies.

The knowledge about the normal distribution of the original fluorescent probe is used to establish which part or which parts of the terminal (or entire) amino-acid sequence that is important for the attachment of this fluorescent probe to subcellular structures, giving it its specific spatially distributed pattern in the cell or cells, when such a pattern has been established as the normal distribution of this fluorescent probe. This may be accomplished by creating new fluorescent probes where a systematic deletion of short N- or C-terminal or internal sequences (number of DNA bases) of the original fluorescent probe are made. These new shorter variants of the of the original fluorescent probe construct are transfected into the cells of interest and then the cells are examined for spatial distribution of the new fluorescent probes as described above for the original fluorescent probe. In those cells where the new fluorescent probe distribution pattern is different from the original fluorescent probe distribution pattern it is evident that part of the, or the entire, targeting sequence has been deleted. The DNA- or amino-acid sequence of the missing part therefore contains the structural information necessary for association of the original fluorescent probe with its intracellular binding sites.

Peptides for inhibition of the established normal distribution of the original fluorescent probe are designed according to the hypothesis, that the deduced targeting sequence, or sequences, in the original fluorescent probe amino-acid sequence are the important sequences for the actual spatial distribution of the original fluorescent probe in intact living cells, is tested. This is done by producing peptides of identical amino-acid sequence as the deduced targeting sequence or parts thereof and introducing them into the cytoplasm, either by microinjection or transient or permanent permeabilisation, of cells containing the original fluorescent probe and thereafter monitoring the spatial distribution of the original fluorescent probe in the cells. If the deduced targeting sequence or sequences are of importance for the actual spatial distribution of the original fluorescent probe in intact living cells, the introduced peptides will self-associate with the anchoring sites for the original fluorescent probe and thereby disrupt the normal distribution of the original fluorescent probe. In order to have this effect, the introduction of the peptides should change the original distribution pattern so that a decrease in fluorescence of 10% or more, compared to the pattern before their introduction, can be

detected. This is done by observing the same cells before and after administration of the peptides. When peptides that fulfil this criterion have been found they are called 'peptide leads' and will hereafter be referred to using this expression. These peptide leads can now be used as a basis for the design of organic molecules that can be used eventually to disrupt the spatial distribution of the original fluorescent probe but also as control compounds in screening assays.

PS473 and derivatives thereof show a discrete intracellular localisation that allow establishment of assay systems valuable in the screening for compounds that modulate targeting of said probes. IKKβ interacts with multiple components of the IkappaB complex. Construction of the described assay systems has allowed us to screen for compounds that interact with specific or multiple targeting sites. This approach allow for development of compounds that through modulation of one (or several) of multiple targeting sites of IKKβ (or other IKKs) will provoke either a partial or a complete inhibition of the NF-kappaB activation. In addition cell specific anchoring will allow design of compounds that only affect defined cell types.

In parallel to the above mentioned step wherein peptide leads are defined, the distribution pattern found for the original fluorescent probe is compared to the naturally occurring spatial distribution of the protein on which the original fluorescent probe is based. This may be accomplished by observing fixed primary cells separated from or still within the tissue of interest and fixed cells that contain the original fluorescent probe. Thereafter the protein is stained using ordinary immunocytochemical or immunohistochemical methods and the spatial distribution revealed by this staining procedure is compared to the spatial distribution of the original fluorescent probe. It is desirable, but not required, that a high degree of correlation between the two patterns obtained in this step can be observed.

Establishment of a primary screening assay is normally done by making use of the cells of interest containing the original fluorescent probe as the basis for a screening assay. Depending on the knowledge acquired about the behaviour of the original fluorescent probe when subjecting the cells to physiologically relevant influences the assay procedure can be chosen: 1. If the fluorescent probe normally is targeted to specific sites and stays associated with these sites during stimulation of the intracellular pathway, the assay should preferably be designed to detect dislocation of the original fluorescent

probe from the targeting sites in mechanically intact or permeabilised living cells. This is an assay where the dislocation can be detected within minutes after application of an influence and the time frame for the detection and time for exposing the cells to an influence should be chosen to match this. 2. If the desire is to disrupt the actual targeting event rather than dislocate already targeted fluorescent probe the influence may need hours to produce a detectable response. The actual measurement, still of a change in the fluorescence or luminescence distribution pattern compared to the normal distribution pattern for the original fluorescent probe, may be made at two time points; before and after the influence has exerted any effect it may have. This is an assay where the effect of an influence may require several hours to produce a detectable response and the time frame for the detection and time for exposing the cells to an influence should be chosen to match this. 3. If the fluorescent probe normally redistributes between two intracellular sites upon activation of the intracellular pathway one may either want to disrupt the initial targeting or dislocate the original fluorescent probe from its initial or resting anchoring site. In this case procedure no. 1 above may be used. If the desire instead is to inhibit the association of the original fluorescent probe with the site it redistributes to during activation of the intracellular pathway the targeting sequence of this site should be in focus for the lead peptide generation. This is an assay where the redistribution may be detected within minutes after application of an influence and the time frame for the detection and time for exposing the cells to an influence should be chosen to match this. Furthermore, any influence applied to inhibit the targeting of the original fluorescent probe upon its redistribution may need to be added to the cells before activation of the intracellular pathway.

While the original fluorescent probe and peptide leads will be used in the actual primary screening assay, it is also desirable to have a counterscreen or counterscreens directed at protein isoforms that one does not wish to affect. In order to accomplish this, constructs are made for new fluorescent probes encoding the protein isoforms tagged with GFP. These constructs are subsequently transfected into the cells of interest. When the new fluorescent probes are expressed in the cells, some of the cells are chosen as the basis for new cell lines that can be used in the counterscreen or counterscreens.

Suitable probes for this purpose comprise DNA constructs encoding fusion polypeptides comprising forms of IKKα, IKKβ, IKKγ or NIK and GFP; PDE1, PDE2, PDE3, PDE4, PDE5, PDE6, PDE7, PDE8 , PDE9 or PDE10 and GFP; PKA catalytic subunit and GFP.

In a preferred embodiment the DNA constructs will encode fusion polypeptides comprising isoforms of IKKβ, PDE 4, mPDE5, PKA catalytic subunit and GFP.

In a much preferred embodiment the DNA construct is selected from table 1.

Table 1 list of the fusion constructs of the invention by the names used herein as well as by reference to relevant SEQ ID NOs of sequences of DNA encoding the construct and full amino acid sequences

Fusion construct	DNA sequence SEQ ID NO:	Protein Sequence SEQ ID NO:
PDE 4D3 - EGFP	1	2
PDE 4D4 - EGFP	3	4
PDE 4D5 - EGFP	5	6
PDE 5 - EGFP	7	8
IKKβ - EGFP	9	10
NF-KappaB - EGFP	11	12
EGFP - IKKβ	13	14
EGFP - IKKβL2	15	16

The cell lines established for the primary screen and the counterscreen, or counterscreens, are used to establish peptide leads that more specifically dislocate the desired isoform of the protein of interest compared to other isoforms of the same protein. The peptide leads are introduced into the cells as described above and the changes in spatial distribution of the original and counterscreen fluorescent probes are quantified and dose-response relationships are established for each lead peptide. Thereafter the dose-response relationships are compared. A peptide lead is considered specific for the original fluorescent probe if the dose of the peptide required to dislocate at least 10% of the fluorescent probes in the counterscreen or conterscreens are at least two times higher than the dose required to dislocate 10% of the original fluorescent probe. The lead peptides with the biggest dose difference when comparing the primary and the counterscreen dose-response relationships are chosen as the basis for the next step in the discovery project.

In one embodiment the primary screening assay and counterscreen or counterscreens are used to define specificity of the peptide leads by using a procedure that compares their ability to cause a dislocation, disruption of targeting or inhibition of redistribution of the original fluorescent probe in the primary screening assay to their ability to cause a

dislocation, disruption of targeting or inhibition of redistribution of the new fluorescent probes in the counterscreen or counterscreens.

In a preferred embodiment the dose of a peptide lead required to cause a quantified dislocation, disruption of targeting or inhibition of redistribution of the original fluorescent probe of at least 10% in the primary screening assay is 50% or less of the dose required to cause a quantified dislocation, disruption of targeting or inhibition of redistribution of the new fluorescent probes of at least 10% in the counterscreen or counterscreens.
The invention provides for a specificity index which may be constructed describing a numerical relationship, with the primary screening asay result first, of the dose required to produce half-maximal effect in the primary assay compared to the dose required to produce half-maximal effect in the counterscreen or counterscreens.
In one embodiment the peptide leads chosen for further use in the discovery project have a specificity index of 1 to 2.
In another embodiment the peptide leads chosen for further use in the discovery project have a specificity index between 1 to 2 and 1 to 10.
In a further embodiment the peptide leads chosen for further use in the discovery project have a specificity index between 1 to 11 and 1 to 100.
In yet a further preferred embodiment the peptide leads chosen for further use in the discovery project have a specificity index better than 1 to 100.

Lead peptides are used to create and select libraries of small organic molecules that can be useful in screening assays to find bioactive substances useful as drugs to treat the condition or disease of interest for the project. In this step the amino-acid sequence information and other structural information about the lead peptide or peptides is used to extract information useful for finding and/or defining and synthesising bioactive organic molecules that can mimic the effect of the lead peptides on the normal spatial distribution pattern of the original fluorescent probe. Such compounds may be useful as drugs to treat the condition or disease of interest for the project. Peptide leads selected by the discovery project are used to design and assemble compound libraries based on the structural and chemical information inherent in the lead peptides using prior chemical knowledge and computational chemistry approaches so that the compounds have a structure that give them the ability to interact with or bind to the targeting sequence of IKKβ, PDE 4D X or mPDE5 thereafter testing the compound libraries at a concentration of 10 or 100 micromolar of each compound in the primary screening assay.

When the libraries of compounds have been defined and are at hand it is time to initiate primary screening. In this procedure, cells containing the original fluorescent probe are contacted with the compounds. The compounds are all tested at just one or a few concentrations, typically 10 and 100 micromolar, in a highly parallel fashion using a quantitative fluorescence redistribution assay. Compounds that cause a change in the quantitated response (the response scale defined by the range 0 (no change in redistribution) – 100%) of the assay by more than a predetermined value, typically between 10 and 100%, are considered to be "primary hits". The primary hits are then further characterised: 1. for potency by establishing a dose-response relationship compared to the lead peptide(s) using the primary screening assay 2. for selectivity by establishing a dose-response relationship in the counterscreen or counterscreens. Primary hits that have low potency, typically when the half-maximal effect of the compound in the primary assay is achieved at a concentration of the compound between 10 and 100 micromolar, may not need testing in the counterscreen or counterscreens since the likelihood that they will be used beyond this step in the discovery project is small. Primary hits that have equal or lower potency in the primary screening assay compared to the counterscreen or counterscreens are regarded as non-selective and the likelihood that they will be used beyond this step in the discovery project is small. Primary hits that display some degree of selectivity, typically half maximal effect in the primary screening assay at a concentration 50% or less of the concentration that gives half maximal effect in the counterscreen or counterscreens are considered interesting as the basis for further chemical synthesis or construction of new libraries of compounds and will hereafter be referred to as "primary lead compounds".

Compounds that cause a change in the quantitated response, with a response scale from 0 to 100% based on the absence of a response and the maximal response observed with the peptide leads in the primary screening assay, of the assay by more than a predetermined value are selected and called "primary hits".

In one embodiment the predetermined value is 10%.

In another embodiment the predetermined value is 50%.

In yet another embodiment the predetermined value is 70%.

In one embodiment the primary hits are further characterised for potency and maximal effect by establishing a dose-response relationship and comparing that to the effects of the lead peptides using the primary screening assay and for selectivity by establishing a dose-response relationship in the counterscreen or counterscreens.

Primary hits may be deselected by the discovery project when they display a half-maximal potency at a dose corresponding to a concentration of more than 10 micromolar or because they display a selectivity index less than 1 to 2.
Primary hits may be selected by the discovery project when they display a half-maximal potency at a dose corresponding to a concentration of 10 micromolar or less or because they display a selectivity index higher than 1 to 2, the compounds hereafter also referred to as "primary lead compounds".

A Structure-Activity Relationship (SAR) is built by iterations of compound library composition and screening to define drug candidate leads. This step is included to further improve the possibilities of finding bioactive compounds with desirable properties for treatment of the diseases or conditions of interest to the project. The primary lead compounds are here used to provide chemical structural information that can be used as the basis for composition or chemical synthesis of new, directed, compound libraries. By systematic chemical modification of part of the structure of one or more primary lead compounds new libraries are assembled. These new libraries of compounds are also investigated using the primary screening assay and counterscreen or counterscreens. Preferably, dose-response relationships are recorded for each chemical modification of the primary lead compound and compared to the primary lead compound itself. Thereby SAR is established. Among the new compounds, the ones that in this step has the best combination of potency and specificity are chosen either as the basis for a new round of compound library synthesis or composition or, as the final step of the SAR building process, as compounds that will be further for actual pharmacoloical effects in assay systems and animals that are relevant to the underlying physiological and pathophysiological processes of interest to the project. The latter compounds will hereafter be referred to as "drug candidate leads".
In one embodiment drug candidate leads have a half-maximal potency at a dose corresponding to a concentration of less than 1 micromolar and a selectivity index higher than 1 to 2.
In one embodiment the drug candidate leads have a half-maximal potency at a dose corresponding to a concentration of less than 1 micromolar and a selectivity index higher than 1 to 10.
In one embodiment the drug candidate leads have a half-maximal potency at a dose corresponding to a concentration of less than 1 micromolar and a selectivity index higher than 1 to 100.

In one embodiment the drug candidate leads have a half-maximal potency at a dose corresponding to a concentration of less than 0,1 micromolar and a selectivity index higher than 1 to 2.
In a preferred embodiment the drug candidate leads have a half-maximal potency at a dose corresponding to a concentration of less than 0,1 micromolar and a selectivity index higher than 1 to 10.
In another preferred embodiment the drug candidate leads have a half-maximal potency at a dose corresponding to a concentration of less than 0,1 micromolar and a selectivity index higher than 1 to 100.

Drug candidate leads may be further characterised in tissue based, cell based and biochemical assays to validate *in vitro* their efficacy and toxicity. There are many ways to test efficacy of a drug candidate lead. Preferably, the drug candidate lead is tested in assay systems with high relevance to the underlying physiological and pathophysiological processes involved in the pathogenesis and pathophysiology of the disease or condition of interest to the project. Likewise, the drug candidate leads are tested for toxic effects, preferably testing for genetic effects (influence on the integrity and arrangement of DNA), metabolic effects (influence on cellular metabolic processes) and cytotoxic effects (influence on cell integrity and organelle integrity). There is a high likelihood that drug candidate leads, that do not show appropriate efficacy or that display toxicity will not be used beyond this step in the discovery project because it is expected that such compounds are less suitable as actual drugs to be used in an animal.
In one embodiment drug candidate leads chosen by the discovery project are tested *in vitro* for efficacy, in assay systems with high degree of relevance to the underlying physiological and patophysiological processes involved in hypotension, inflammatory diseases, and for toxicity, preferably testing for genetic, metabolic and cytotoxic effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity are chosen to be the candidates that will enter testing in animals.
In another embodiment drug candidate leads chosen by the discovery project are tested *in vitro* for efficacy, in assay systems with high degree of relevance to the underlying physiological and patophysiological processes involved in inflammatory airway diseases, and for toxicity, preferably testing for genetic, metabolic and cytotoxic effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity are chosen to be the candidates that will enter testing in animals.

In another embodiment drug candidate leads chosen by the discovery project are tested *in vitro* for efficacy, in assay systems with high degree of relevance to the underlying physiological and patophysiological processes involved in inflammatory joint diseases, and for toxicity, preferably testing for genetic, metabolic and cytotoxic effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity are chosen to be the candidates that will enter testing in animals.
In another embodiment drug candidate leads chosen by the discovery project are tested *in vitro* for efficacy, in assay systems with high degree of relevance to the underlying physiological and patophysiological processes involved in inflammatory bowel diseases, and for toxicity, preferably testing for genetic, metabolic and cytotoxic effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity are chosen to be the candidates that will enter testing in animals.
In another embodiment drug candidate leads chosen by the discovery project are tested *in vitro* for efficacy, in assay systems with high degree of relevance to the underlying physiological and patophysiological processes involved in autoimmune diseases, and for toxicity, preferably testing for genetic, metabolic and cytotoxic effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity are chosen to be the candidates that will enter testing in animals.

In a preferred embodiment of the present invention I-kappaB degradation is inhibited by a novel mechanism namely by mis-targeting and/or modulation of the redistribution of specific IKKs. In contrast to previous interventions involving IKK the presented invention does not involve direct inhibition of the IKK enzymatic activity.

This completely novel mechanism for inhibition of the overall effect of the IKK complex provides clear advantages as it opens for a higher IKK isoform selectivity and a higher cell specificity of the therapy. In addition cell specific anchoring will allow design of compounds that only affect defined cell types.

In one aspect of the invention the substance is an organic compound, the organic compound being a weak acid in that it is a neutral molecule that can reversibly dissociate into an anion (a negatively charged molecule) and a proton (a hydrogen ion). In another aspect, the organic compound is a weak base in that it is a neutral molecule that can form a cation (a positively charged molecule) by combining with a proton. The functional groups of the targeting sequences include functional groups selected from the group

consisting of: methyl-, isopropyl-, isobutyl-, hydroxyl-, thiol-, benzyl-, benzyloyl-, methylindolyl-, methylimidazolyl-, amine-, imine-, carboxyl- and acetamide-groups as parts of amino acids in the targeting sequences.

In another aspect of the invention the organic compound is a compound having one or more chemical domains capable of interacting with one or more functional groups of the targeting sequence of the native anchoring site of the cyclic nucleotide phosphodiesterase or I-kappaB kinase. In yet another aspect the organic compound is a compound having at least two chemical domains capable of interacting with at least two functional groups of the targeting sequence of the native anchoring site for the cyclic nucleotide phosphodiesterase or I-kappaB kinase. In a further aspect the organic compound is a compound having at least three chemical domains capable of interacting with at least three functional groups of the targeting sequence of the native anchoring site for the cyclic nucleotide phosphodiesterase or I-kappaB kinase.

The organic compound is, in one aspect of the invention, a compound having at least two chemical domains capable of interacting with at least two functional groups of the targeting sequence of the cyclic nucleotide phosphodiesterase. In a specific embodiment, the organic compound is a compound having at least three chemical domains capable of interacting with at least three functional groups of the targeting sequence of the cyclic nucleotide phosphodiesterase.

In the next part of the discovery process the drug candidate leads are tested *in vivo* for toxic and unwanted effects in animals such as mice and rats. The drug candidate leads are also tested for efficacy in animals that have a disease or condition with high degree of relevance to the disease or condition of interest to the project. The drug candidate leads may also be tested for efficacy in animals which have been treated in a way that make them experience a disease or condition with high degree of relevance to the disease or condition of interest to the project. Drug candidate leads that display efficacy in one or more of such animal tests and that does not display any apparent toxicity at a dosage level, preferably 2 –10 times higher than the level that gives satisfactory efficacy are chosen to be the final drug candidates that should be considered for further animal testing and initial testing in humans. These compounds are hereafter referred to as "discovery project leads".

In one embodiment drug candidate leads chosen by the discovery project are tested *in vitro* for efficacy, in assay systems with high degree of relevance to the underlying physiological and pathophysiological processes involved in depression, and for toxicity, preferably testing for genetic, metabolic and cytotoxic effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity are chosen to be the candidates that will enter testing in animals.

In another embodiment drug candidate leads chosen by the discovery project are tested *in vitro* for efficacy, in assay systems with high degree of relevance to the underlying physiological and pathophysiological processes involved in jet-lag, and for toxicity, preferably testing for genetic, metabolic and cytotoxic effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity are chosen to be the candidates that will enter testing in animals.

In another embodiment drug candidate leads chosen by the discovery project are tested *in vitro* for efficacy, in assay systems with high degree of relevance to the underlying physiological and patophysiological processes involved in erectile dysfunction, and for toxicity, preferably testing for genetic, metabolic and cytotoxic effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity are chosen to be the candidates that will enter testing in animals.

In one embodiment drug candidate leads chosen by the discovery project are tested for efficacy, in healthy animals and animals with a condition with high degree of relevance to the underlying physiological and pathophysiological processes involved in hypotension, and for toxicity and unwanted side effects, after which the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity or unwanted side effects are chosen to be the candidates, called discovery project leads, that will enter further testing in animals and testing in humans.

In one embodiment drug candidate leads chosen by the discovery project are tested for efficacy, in healthy animals and animals with a condition with high degree of relevance to the underlying physiological and pathophysiological processes involved in inflammatory diseases, and for toxicity and unwanted side effects, after which the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity or unwanted side effects are chosen to be the candidates, called discovery project leads, that will enter further testing in animals and testing in humans.

In one embodiment drug candidate leads chosen by the discovery project are tested for efficacy, in healthy animals and animals with a condition with high degree of relevance to the underlying physiological and pathophysiological processes involved in hypertension,

and for toxicity and unwanted side effects, after which the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity or unwanted side effects are chosen to be the candidates, called discovery project leads, that will enter further testing in animals and testing in humans.

In one embodiment drug candidate leads chosen by the discovery project are tested for efficacy, in healthy animals and animals with a condition with high degree of relevance to the underlying physiological and pathophysiological processes involved in jet-lag and circadian rhythm resetting, and for toxicity and unwanted side effects, after which the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity or unwanted side effects are chosen to be the candidates, called discovery project leads, that will enter further testing in animals and testing in humans.

In one embodiment drug candidate leads chosen by the discovery project are tested for efficacy, in healthy animals and animals with a condition with high degree of relevance to the underlying physiological and pathophysiological processes involved in erectile dysfunction, and for toxicity and unwanted side effects, after which the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity or unwanted side effects are chosen to be the candidates, called discovery project leads, that will enter further testing in animals and testing in humans.

In one embodiment drug candidate leads chosen by the discovery project are tested for efficacy, in healthy animals and animals with a condition with high degree of relevance to the underlying physiological and pathophysiological processes involved in inflammatory airway diseases, and for toxicity and unwanted side effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity or unwanted side effects are chosen to be the candidates, called discovery project leads, that will enter further testing in animals and testing in humans.

In one embodiment drug candidate leads chosen by the discovery project are tested for efficacy, in healthy animals and animals with a condition with high degree of relevance to the underlying physiological and pathophysiological processes involved in inflammatory joint diseases, and for toxicity and unwanted side effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity or unwanted side effects are chosen to be the candidates, called discovery project leads, that will enter further testing in animals and testing in humans.

In one embodiment drug candidate leads chosen by the discovery project are tested for efficacy, in healthy animals and animals with a condition with high degree of relevance to

the underlying physiological and pathophysiological processes involved in inflammatory bowel diseases, and for toxicity and unwanted side effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity or unwanted side effects are chosen to be the candidates, called discovery project leads, that will enter further testing in animals and testing in humans.

In one embodiment drug candidate leads chosen by the discovery project are tested for efficacy, in healthy animals and animals with a condition with high degree of relevance to the underlying physiological and pathophysiological processes involved in autoimmune diseases, and for toxicity and unwanted side effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity or unwanted side effects are chosen to be the candidates, called discovery project leads, that will enter further testing in animals and testing in humans.

In one embodiment drug candidate leads chosen by the discovery project are tested for efficacy, in healthy animals and animals with a condition with high degree of relevance to the underlying physiological and pathophysiological processes involved in depression, and for toxicity and unwanted side effects, whereafter the drug candidate leads that display the best efficacy and the least, or no, indications of toxicity or unwanted side effects are chosen to be the candidates, called discovery project leads, that will enter further testing in animals and testing in humans.

The administration route of any of the compounds of the invention may be of any suitable route which leads to a concentration in the blood corresponding to a therapeutic concentration by the oral route, the parenteral route, the cutaneous route, the nasal route, the rectal route, the vaginal route and the ocular route. It should be clear to a person skilled in the art that the administration route is dependant on the compound in question, particularly, the choice of administration route depends on the physico-chemical properties of the compound together with the age and weight of the patient and on the particular disease and the severity of the same.

The compounds of the invention may be contained in any appropriate amount in a pharmaceutical composition, and are generally contained in an amount of about 1-95% by weight of the total weight of the composition. The composition may be in form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, delivery devices, suppositories, enemas, injectables, implants, sprays, aerosols and in other suitable form. The pharmaceutical compositions may be formulated according to conventional

pharmaceutical practice, see, e.g., "Remington's Pharmaceutical Sciences" and "Encyclopedia of Pharmaceutical Technology".

Pharmaceutical compositions according to the present invention may be formulated to release the active compound substantially immediately upon administration or at any substantially predetermined time or time period after administration. The latter type of compositions are generally known as controlled release formulations. Controlled release formulations may also be denoted "sustained release", "prolonged release", "programmed release", "time release", "rate-controlled" and/or "targeted release" formulations.

In the present context every pharmaceutical composition is an actual drug delivery system, since upon administration it presents the active drug substance to the body of the organism.

The compounds of the invention are preferably administered in an amount of about 0.1-30 mg per kg body weight per day, such as about 0.5-15 mg per kg body weight per day. The compound in question may be administered orally in the form of tablets, cap-sules, elixirs or syrups, or rectally in the form of suppositories. Parenteral administration of the compounds of the invention, is suitably performed in the form of saline solutions of the compounds or with the compound incorporated into liposomes. In cases where the compound in itself is not sufficiently soluble to be dissolved, an acid addition salt of a basic compound can be used, or a solubilizer such as ethanol can be applied.

Oral administration. For compositions adapted for oral administration for systemic use, the dosage is normally 1 mg to 1 g per dose administered 1-4 times daily for 1 week, 12 months or even lifelong depending on the disease to be treated.

Rectal administration. For compositions adapted for rectal a somewhat higher amount of compound is usually preferred, i.e. from approximately 1 mg to 100 mg per kg body weight per day.

Parenteral administration. For parenteral administration a dose of about 0.1 mg to about 50 mg per kg body weight per day is convenient. For intravenous administration a dose of about 0.1 mg to about 20 mg per kg body weight per day. For intraarticular administration a dose of about 0.1 mg to about 20 mg per kg body weight per day is usually preferable. For parenteral administration in general, a solution in an aqueous medium of 0.5-2% or more of the active ingredients may be employed.

Cutaneous administration. For topical administration on the skin a dose of about 1 mg to about 5 g administered 1-10 times daily is usually preferable.

EXAMPLES

Example 1: Probes for detection of PDE4D dislocation.

These are specific PDE4D variants fused to a GFP. Currently 5 PDE4D splice variants are known: PDE4D1, PDE4D2, PDE4D3, PDE4D4 and PDE4D5. These all share C-terminal sequences but differ in their N-termini.

Inspection of the scientific litterature indicates that the PDE4D1 and PDE4D2 subtypes are found only in the cytosolic fraction, whereas PDE4D3, PDE4D4 and PDE4D5 subtypes appear to associate with some form of cellular structure(s). Targetting sequences of PDE4Ds are presently believed to be located in their N-terminal domain(s). In accordance with this, PDE4D1 and PDE4D2 have much shorter N-terminal domains than PDE4d3, PDE4D4 and PDE4D5. To best preserve the normal distribution of PDE4Ds, the fusions are made between the C-terminus of the PDE4D species and the N-terminal of the GFP.

To construct PDE4D-GFP fusions, PDE4D sequences are amplified using PCR according to standard protocols with specific top-primers as listed below, and the common bottom-primer listed below. The PCR products are digested with restriction enzymes Hind3 and EcoR1, and ligated into pEGFP-N1 (Clontech, Palo Alto; GenBank Accession number U55762) digested with Hind3 and EcoR1. This produces PDE4D-EGFP fusions under the control of a CMV promoter (SEQ ID NOs: 5 and 6 (PDE4D5-EGFP); SEQ ID NOs: 3 and 4 (PDE4D4-EGFP); SEQ ID NOs: 1 and 2 (PDE4D3-EGFP)).

Top primers all include specific sequences following the ATG, a Kozak sequence, and a cloning site (Hind3). The bottom primer includes the common C-terminal sequence minus the stop codon, an EcoR1 cloning site, and an extra nucleotide to preserve the reading frame in EGFP-N1.

Sequences of top-primers:

5'-GTAAGCTTCGAACATGATGCACGTGAATAATTTTCCC-3' ; specific for PDE4D3A and PDE4D3B (GenBank Acc. nos. L20970 & U50159).

5'-GTAAGCTTCGAACATGGAGGCAGAGGGCAGCAGC-3'; specific for PDE4D4A (GenBank Acc. no. L20969).

5'-GTAAGCTTCGAACATGGCTCAGCAGACAAGCCCG-3'; specific for PDE4D5A (GenBank Acc. no. AF012073).

Sequence of common bottom-primer:
5'-GTGAATTCCCGTCGTGTCAGGAGAAGCATCATCTATG-3'.

The resulting plasmids are transfected into a suitable cell line, e.g. MVLEC. The subcellular distribution of the probes is examined carefully by fluorescence microscopy, both under resting conditions, and upon elevation of cAMP, e.g. by activation of adenylate cyclase with forskolin, which may or may not have an effect on the normal distribution.

Example 2: Probes for detection of PDE5 dislocation:

These are specific PDE5 variants fused to a GFP. Currently only one main human variant is known (GenBank Acc.nos. AJ004865 and D89094).
Inspection of the scientific litterature indicates that the catalytic domain is contained in the C-terminal part of the protein, so potential targeting sequences of PDE5 may be located in the N-terminal part. To best preserve the normal distribution of PDE5, the first fusion is made between the C-terminus of the PDE5 species and the N-terminal of the GFP.
To construct the PDE5-GFP fusions, PDE5 sequences are amplified using PCR according to standard protocols with the specific primers listed below. The PCR product is digested with restriction enzymes EcoR1 and Acc65I, and ligated into pEGFP-N1 (Clontech, Palo Alto; GenBank Accession number U55762) digested with EcoR1 and Acc65I. This produces a PDE5-EGFP fusion under the control of a CMV promoter (SEQ ID NOs: 7 and 8).
The top primer includes specific sequences following the ATG, a Kozak sequence, and a cloning site (EcoR1). The bottom primer includes specific C-terminal sequences minus the stop codon, an Acc65I cloning site, and two extra nucleotides to preserve the reading frame in EGFP-N1.

PDE5-top :
5'-GTGAATTCAACCATGGAGCGGGCC-3'

PDE5-bottom:
5'-GTGGTACCCAGTTCCGCTTGGCC

The resulting plasmids are transfected into a suitable cell line, e.g. MVLEC. The subcellular distribution of the probes is examined carefully by fluorescence microscopy, both under resting conditions, and upon elevation of cGMP, e.g. by activation of cyclase with NO or nitroprusside, which may or may not have an effect on the normal distribution.

EXAMPLE 3: Probes for detection of IKK redistribution.

Modulation of IKKβ redistribution by mis-targeting provoke an inhibition of cytokine-induced NF-kappaB activation. In the present example it is shown that specific mis-tageting of IKKβ inhibits cytokine-induced NF-kappaB activation. Dislocation of endogenous IKKβ from its anchoring sites is achieved by expression of a C-terminal part of IKKβ (PS473). The PS473 probe, which is a GFP fusion, allows a simultaneous monitoring of its localisation and redistribution.

Expression of the PS473 probe has a clear inhibitory activity on cytokine-induced activation of NF-kappaB. For the first time we hereby show that dislocating IKKβ, without directly affecting its kinase activity, effectively hampers the functional activity of NF-kappaB. This causal relationship between mis-targeting of IKKβ and a lacking NF-kappaB activity is studied in two different systems: a) Real-time measurement of NF-kappaB translocation from the cytoplasm to the nucleus, and b) measurement of NF-kappaB induced transcriptional activity.

These are specific IKK subunit variants fused to a GFP. As examples, the following three subunits have been chosen: IKKα (GenBank Acc.no. AF009225) , IKKβ (GenBank Acc. No. AF031416), IKKγ (GenBank Acc. No. AF074382) and NIK (GenBank Acc. No. NM003954).

Inspection of the scientific literature indicates that IKKβ dissociates transiently from the IKAP complex during activation, and so becomes the first choice for a probe to detect redistribution.

To construct the IKKβ-GFP fusion, IKKβ sequences are amplified using PCR according to standard protocols with the specific primers listed below. The PCR product is digested with restriction enzymes Hind3 and Acc65I, and ligated into pEGFP-N1 (Clontech, Palo Alto; GenBank Accession number U55762) digested with Hind3 and Acc65I. This produces an IKKβ-EGFP fusion under the control of a CMV promoter (SEQ ID NOs: 9 and 10).

The top primer includes specific sequences following the ATG and a cloning site (Hind3). The bottom primer includes specific C-terminal sequences minus the stop codon, an Acc65I cloning site, and two extra nucleotides to preserve the reading frame in EGFP-N1.

IKKβ-top:
5'-GTAAGCTTACATGAGCTGGTCACCTTCCCTG-3'

IKKβ-bottom:
5'-GTGGTACCCATGAGGCCTGCTCCAG-3'

The resulting plasmids are transfected into a suitable cell line. The subcellular distribution of the probes is examined carefully by fluorescence microscopy, both under resting conditions, and upon activation, e.g. with TNFα.

Probes for detection of activation of the NFkappaB signal transduction pathway.

Plasmid PS377 contains an NFkappaBp65-EGFP fusion. The GenBank accession number of the p65 subunit of NFkappaB is M62399. It is constructed by performing PCR on human cDNA (from Clontech) with specific primers p65-top and p65-bottom. The resulting ca. 1.7 kb PCR product is cut with restriction enzymes Xho1 and Hind3 and cloned into pEGFP-N1 (Clontech) cut with Xho1 and Hind3. This produces an NFkappaB-EGFP fusion (SEQ ID NOs: 11 and 12) under the control of the CMV promoter.

p65-top: 5'-TTTTACTCGAGATGGACGAACTGTTCCCCCTCA-3'
p65-bottom: 5'-TTTTGAAGCTTGGAGCTGATCTGACTCAGCAGG-3'

Construction of a reporter gene assay for monitoring NFkappaB-induced transcriptional activation:
Plasmid PS397 contains a selectable NFkappaB reporter construct. It is constructed through ligation of two BamH1-Not1 fragments: A 2.4 kb fragment from pNFkappaB-Luc (from Clontech,), which contains a luciferase gene and NFkappaB response elements, and a 2.8 kb BamH1-Not1 fragment from pZeoSV (from Invitrogen), which contains

essential plasmid elements and a zeocin selective marker for use in E.coli and mammalian cells.

Construction of probes for monitoring IKKβ localisation, mis-targeting and redistribution in live cells:
Plasmid PS410 contains an EGFP-IKKβ fusion. The GenBank accession number of the beta subunit of IkappaB kinase is AF031416. It is constructed by performing PCR on human cDNA (from Clontech) with specific primers IKKβ-top and IKKβ-stop. The resulting 2.2 kb PCR product is cut with restriction enzymes Hind3 and Acc65I and cloned into pEGFP-C1 (Clontech) cut with Hind3 and Acc65I. This produces an EGFP-IKKβ fusion (SEQ ID NOs: 13 and 14) under the control of the CMV promoter.

IKKβ-top: 5'-GTAAGCTTACATGAGCTGGTCACCTTCCCTG-3'
IKKβ-stop: 5'-GTGGTACCTCATGAGGCCTGCTCCAG-3'

Plasmid PS472 contains a full length IKKβ under the control of the CMV promoter. It is constructed by cutting PS410 with restriction enzymes Nhe1 and Hind3, which flank EGFP. This excises EGFP sequences from the plasmid, while placing IKKβ immediately downstream of the CMV promoter. The protruding ends generated by the enzymes are then made blunt using Klenow polymerase according to standard protocol, and the plasmid is recircularized with DNA ligase.

PS473 contains EGFP fused to the C-terminal part of IKKβ. This part of IKKβ contains a putative leucine zipper region, but is without catalytic activity as this function resides in the N-terminal part of IKKβ. It is constructed by performing PCR on PS410 with primers IKKβ-LZ-top and IKKβ-stop. IKKβ-LZ-top contains a Hind3 site and specific IKKβ sequence from amino acid position 455 in the predicted amino acid sequence. This is almost immediately upstream of the first leucine of the predicted leucine zipper, which is at position 458. The resulting 0.9 kb PCR product is cut with restriction enzymes Hind3 and Acc65I and cloned into pEGFP-C1 (Clontech) cut with Hind3 and Acc65I. This produces an EGFP-IKKβ-LZdomain fusion (SEQ ID NOs: 15 and 16) under the control of the CMV promoter.

IKKβ-LZ-top: 5'-GTAAGCTTCCACCATGATGAATCTCCTCCGAAAC-3'

Plasmid PS474 contains the IKKβ C-terminal part under the control of the CMV promoter. It is constructed by cutting PS473 with restriction enzymes Age1 and BspE1, which flank EGFP. This excises EGFP sequences from the plasmid, while placing IKKβ sequences immediately downstream of the CMV promoter. As Age1 and BspE1 produce compatible ends, the plasmid is simply recircularized with DNA ligase. The ATG methionine codon at position 455 in the predicted amino acid sequence of IKKβ, may serve as initiation codon in this construct.

Transfections and cell culture conditions.

Chinese hamster ovary cells (CHO), Human epithelial kidney cells (HEK293) and Human epithelial adenocarcinoma cells (HeLa), were transfected with above mentioned plasmids using FuGENE transfection reagent (Boehringer Mannheim). Stable transfectants were selected using 1000 μg Zeocin/ml (Invitrogen) or 500 μg G418/ml (*Neo* marker) in the growth medium [DMEM (HEK293 and HeLa) or HAM F12 (CHO) with 1000 mg glucose/l, 10 % fetal bovine serum (FBS), 100 μg penicillin-streptomycin mixture ml^{-1}, 2 mM L-glutamine purchased from Life Technologies Inc., Gaithersburg, MD, USA).

For fluorescence microscopy, cells were allowed to adhere to Lab-Tek chambered coverglasses (Nalge Nunc Int., Naperville, IL, USA) for at least 24 hours and cultured to about 80% confluence. Prior to experiments, the cells were cultured over night without selection pressure in DMEM or HAM F-12 medium with glutamax (Life Technologies), 100 μg penicillin-streptomycin mixture ml^{-1} and 0.3 % FBS. This medium has low autofluorescence enabling fluorescence microscopy of cells straight from the incubator.

Microscope imaging of localisation and redistribution in live cells:

Image aquisition of live cells were gathered using a Zeiss Axiovert 135M fluorescence microscope fitted with a Fluar 40X, NA: 1.3 oil immersion objective and coupled to a Photometrics CH250 charged coupled device (CCD) camera. The cells were illuminated with a 100 W HBO arc lamp. For imaging of GFP-based probes we inserted in the light path was a 470±20 nm excitation filter, a 510 nm dichroic mirror and a 515±15 nm emission filter. For imaging of the Hoechst 33342 (H1399, Molecular Probes) nuclear stain we used a 380±20 nm excitation filter, a 410 nm dichroic mirror and a 555±15 nm emission filter

The cells were kept and monitored to be at 37°C with a custom built stage heater.

Quantification of NF-kappaB redistribution:

Cells are stained with the vital nuclear stain, Hoechst.

A sequence of images with a time separation of 10 sec is acquired. At each time point the sequence consists of one NF-kappaB-GFP image and one image of the Hoechst stained nucleus.

The image sequence is corrected for dark current by performing a pixel-by-pixel subtraction of a dark image (an image taken under the same conditions as the actual image, except the camera shutter is not allowed to open).

The image sequence is corrected for non-uniformity of the illumination by performing a pixel-by-pixel ratio with a flat field correction image (an image taken under the same conditions as the actual image of a uniformly fluorescent specimen).

At each time point the accumulated intensity of the NFkappaB probe in the nucleus is ratioed over the total cytoplasmic intensity. The Hoechst image is used to mask the nucleus.

Results:

The full length IKKβ probe (PS410) show an even distribution throughout the cytoplasm when expressed in CHO (Fig. 2) and HEK293 cells. PS473 show a similar localisation after its expression (Fig. 3A). Interestingly however the probe has sensitised the cells to stimuli that induce apoptosis. It is thus observed that the PS473 expressing cells upon 2 hrs of serum starvation undergo apoptosis, in comparison non-tranfected cells or PS410 expressing cells did show no sign on apoptosis after similar treatment. The induction of apoptosis could be visualised as a change in the localisation of the PS473 probe from an even distribution throughout the cytoplasm to a discrete punctate localisation (Fig. 3B).

The PS473 provoked mis-tageting of IKKβ had pronounced functional consequences. We thus observed a prominent inhibition of IL-1 induced NFkappaB redistribution (Fig. 4). Furthermore we observed an inhibition of IL-1 and TNFα induced activation of the NFkappaB regulated transcription as monitored with the above described luciferase reporter construct (PS397) (Fig. 5).

Figur legends

Figure 1

CHO cells expressing PS377 for monitoring NFkappaB redistribution in live cells. A) Before stimulation and B) 10 minutes after stimulation with IL-1 (10 ng/ml).

Figure 2

The full length IKKβ probe (PS410) show an even distribution throughout the cytoplasm when expressed in CHO cells.

Figure 3

PS473 expressed in CHO cells. (A) show an even distribution throughout the cytoplasm. (B) The distributaion change when cells undergo appoptosis as observed after two hours of serum starvation.

Figure 4

Expression of PS473 inhibits IL-1 (0.5 ng/ml) induced redistribution of NF-kappaB in CHO cells.

Figure 5

Expression of PS473 inhibits IL-1 (0.5 ng/ml) and TNF-α (0.5 ng/ml) induced NF-kappaB regulated transcription in HEK293 cells.

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Claims

1. Use of a substance, capable of modulating the specific effectiveness of a cyclic nucleotide phosphodiesterase or I-kappaB kinases through modulating the spatial distribution or change in spatial distribution of the cyclic nucleotide phosphodiesterases or I-kappaB kinases within cells of an animal, for the preparation of a medicament for the prevention or treatment in an animal of an adverse condition which may be reduced or abolished by modulating the activity of one or more cyclic nucleotide phosphodiesterases having the ability to cleave cyclic AMP or cyclic GMP or by modulating the activity of one or more I-kappaB.

2. Use according to claim 1, wherein the I-kappaB kinase is selected from the group consisting of I-kappaB kinase α, I-kappaB kinase β, I-kappaB kinase γ and NIK.

3. Use according to claim 2, wherein the I-kappaB kinase is I-kappaB kinase β.

4. Use according to claim 1, wherein the cyclic nucleotide phosphodiesterase is selected from the group consisting of PDE3, PDE4, PDE7 and PDE8.

5. Use according to claim 4, wherein the cyclic nucleotide phosphodiesterase is PDE4.

6. Use according to claim 5, wherein the cyclic nucleotide phosphodiesterase is a splice variant of PDE4, selected from the group consisting of PDE4A, PDE4B, PDE4C and PDE4D.

7. Use according to claim 6, wherein the PDE4 species is a splice variant of PDE4D.

8. Use according to claim 7, wherein the splice variant is PDE4D1, PDE4D2, PDE4D3, PDE4D4, PDE4D5 and PDE4A1.

9. Use according to claim 8, wherein the splice variant is PDE4D3, PDE4D4 or PDE4D5.

10. Use according to claim 6, wherein the PDE4 splice variant is PDE4A1.

11. Use according to any of the preceding claims, wherein the adverse condition is an inflammatory diseases such as chronic inflammation.

12. Use according to any of claims 1-10, wherein the adverse condition is chronic inflammatory airway diseases such as asthma and chronic bronchial hyperreactivity of non-asthma etiology.

13. Use according to any of claims 1-10, wherein the adverse condition is chronic inflammatory joint diseases such as rheumatoid arthritis and pelvospondylitis.

14. Use according to any of claims 1-10, wherein the adverse condition is chronic inflammatory bowel diseases such as ulcerative colitis and Crohn's disease.

15. Use according to any of claims 1-10, wherein the adverse condition is autoimmune diseases with chronic inflammation such as rheumatoid arthritis, diabetes mellitus type I, systemic lupus erythematosus, myasthenia gravis, Hashimoto's thyreoiditis, Graves' disease and immune thrombocytopenic purpura.

16. Use according to any of claims 1-10, wherein the adverse condition involves a disregulation of the immune system such as acute respiratory distress syndrome (ARDS) and septic shock.

17. Use according to claim 10, wherein the adverse condition is depression.

18. Use according to claim 1, wherein the cyclic nucleotide phosphodiesterase is selected from the group consisting of PDE1, PDE2, PDE5, PDE6, PDE9 and PDE10.

19. Use according to claim 18, wherein the nucleotide phosphodiesterase is a splice variant of PDE5.

20. Use according to claim 18 or 19, wherein the adverse condition is hypo- or hypertension, erectile dysfunction, circadian rhythm resetting or jet-lag.

21. Use according to any of the preceding claims wherein the animal is a mammal.

22. Use according to claim 21, wherein the mammal is a human being.

23. Use according to any of the preceding claims, wherein the substance is an organic compound having a molecular weight of around 3000 Da

24. Use according to any of claims 1-22, wherein the substance is an organic compound having a molecular weight of at the most 1200 Da.

25. Use according to claim 24, wherein the substance is an organic compound having a molecular weight of at the most 900 Da.

26. Use according to claim 25, wherein the substance is an organic compound having a molecular weight of at the most 600 Da.

27. Use according to claim 26, wherein the substance is an organic compound having a molecular weight of at the most 300 Da.

28. Use according to any of the preceding claims, wherein the substance is a peptide.

29. Use according to any of claim 1-27, wherein the substance is a carbon-containing non-peptide.

30. Use according to any of the preceding claims, wherein the organic compound is a compound having one or more chemical domains capable of interacting with one or more functional groups of the targeting sequence of the native anchoring site of the cyclic nucleotide phosphodiesterase or I-kappaB kinase.

31. Use according to any of the preceding claims, wherein the substance interacts with the targeting sequence or part thereof in a manner that dislocates, disrupts targeting, or interferes with redistribution of the fluorescent probe as measured in quantitative fluorescence redistribution assay.

32. A method for extracting quantitative information relating to an influence on a cellular response, the method comprising recording variation, caused by the influence on a mechanically intact living cell or mechanically intact living cells, in spatially distributed

light emitted from a luminophore, the luminophore being part of a fluorescent probe further comprising at least a part of a cyclic nucleotide phosphodiesterase or I-kappaB kinase, the fluorescent probe being present in the cell or cells and being capable of being redistributed in a manner which is related with the degree of the influence, and/or of being modulated by a component which is capable of being redistributed in a manner which is related to the degree of the influence, the association resulting in a modulation of the luminescence characteristics of the luminophore, and processing the recorded variation in the spatially distributed light to provide quantitative information correlating the spatial distribution to the degree of the influence on the cellular response.

33. A screening assay for carrying out the method of claim 32.

34. A screening assay according to claim 32 or 33 wherein the fluorescent probe is modified in a systematic way, still keeping the GFP coding sequence intact, so that the new fluorescent probes are fusion polypeptides where parts of the suspected targeting sequences are altered.

35. A screening assay according to claim 34, wherein the modification of the suspected targeting sequence is a deletion.

36. A screening assay according to any of claims 33-35, wherein the spatial distribution of the fluorescent probe is compared to the spatial distribution of the unmodified fluorescent probe to deduce the targeting sequence.

37. A screening assay according to any of claims 33-36, wherein the quantitative fluorescence redistribution assay is a primary screening assay used in a discovery project

38. A nucleotide sequence encoding the protein corresponding to amino acids 331-552 of SEQ ID NO: 16 or any sub-sequence thereof of more than 25 contiguous amino acids, able to dislocate IKKβ when expressed in CHO cells under the control of the CMV promoter.

39. A nucleotide sequence according to claim 38, wherein the sub-sequence is the predicted leucine zipper contained in amino acids 331-360 of SEQ ID NO: 16.

40. A screening assay according to any of claims 33-37, wherein the fluorescent probe comprises a nucleotide sequence according to claim 38 or 39.

41. A method according to claim 32 wherein the fluorescent probe is able to dislocate IKKβ when expressed in CHO cells under the control of the CMV promoter.

42. A method for preventing or treating, in an animal in need thereof, an adverse condition which may be reduced or abolished by modulating the activity of one or more cyclic nucleotide phosphodiesterases having the ability to cleave cyclic AMP, or cyclic AMP, or by modulating the activity of one or more I-kappaB kinases, the method comprising modulating the specific effectiveness of the cyclic nucleotide phosphodiesterase or I-kappaB kinase by modulating the spatial distribution within cells of the animal.

Figures

Fig. 1A

Fig. 1B

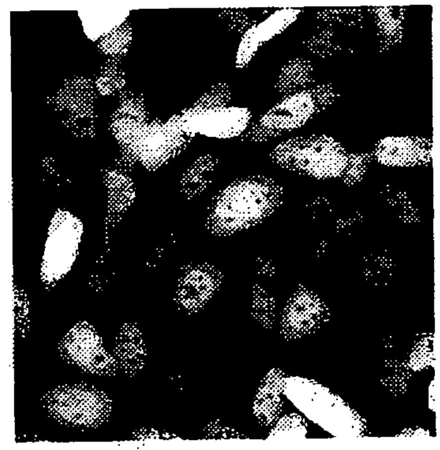


Fig. 2

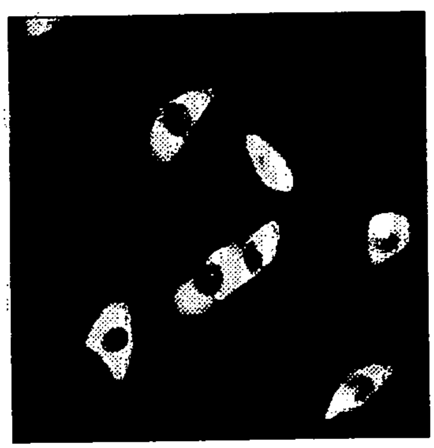


Fig. 3A

Fig. 3B

Fig. 4

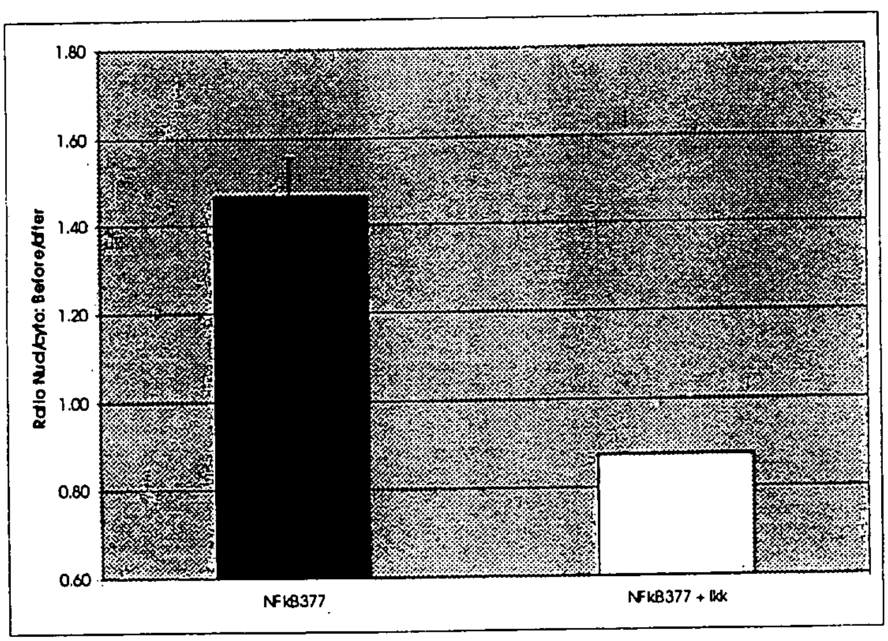
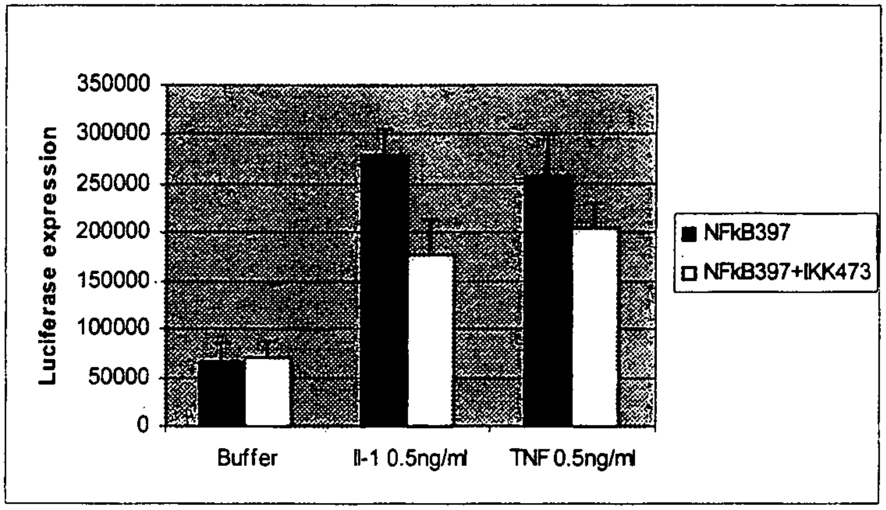


Fig. 5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                325                 330                 335

ttc tta gat aag caa cat gaa gtg gaa att cct tct cca act cag aag     1056
Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr Gln Lys
            340                 345                 350

gaa aag gag aaa aag aaa aga cca atg tct cag atc agt gga gtc aag     1104
Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly Val Lys
        355                 360                 365

aaa ttg atg cac agc tct agt ctg act aat tca agt atc cca agg ttt     1152
Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro Arg Phe
    370                 375                 380

gga gtt aaa act gaa caa gaa gat gtc ctt gcc aag gaa cta gaa gat     1200
Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu Glu Asp
385                 390                 395                 400

gtg aac aaa tgg ggt ctt cat gtt ttc aga ata gca gag ttg tct ggt     1248
Val Asn Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu Ser Gly
                405                 410                 415

aac cgg ccc ttg act gtt atc atg cac acc att ttt cag gaa cgg gat     1296
Asn Arg Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu Arg Asp
            420                 425                 430

tta tta aaa aca ttt aaa att cca gta gat act tta att aca tat ctt     1344
Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr Tyr Leu
        435                 440                 445

atg act ctc gaa gac cat tac cat gct gat gtg gcc tat cac aac aat     1392
Met Thr Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His Asn Asn
    450                 455                 460

atc cat gct gca gat gtt gtc cag tct act cat gtg cta tta tct aca     1440
Ile His Ala Ala Asp Val Val Gln Ser Thr His Val Leu Leu Ser Thr
465                 470                 475                 480

cct gct ttg gag gct gtg ttt aca gat ttg gag att ctt gca gca att     1488
Pro Ala Leu Glu Ala Val Phe Thr Asp Leu Glu Ile Leu Ala Ala Ile
                485                 490                 495

ttt gcc agt gca ata cat gat gta gat cat cct ggt gtg tcc aat caa     1536
Phe Ala Ser Ala Ile His Asp Val Asp His Pro Gly Val Ser Asn Gln
            500                 505                 510

ttt ctg atc aat aca aac tct gaa ctt gcc ttg atg tac aat gat tcc     1584
Phe Leu Ile Asn Thr Asn Ser Glu Leu Ala Leu Met Tyr Asn Asp Ser
        515                 520                 525

tca gtc tta gag aac cat cat ttg gct gtg ggc ttt aaa ttg ctt cag     1632
Ser Val Leu Glu Asn His His Leu Ala Val Gly Phe Lys Leu Leu Gln
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530 535 540

gaa gaa aac tgt gac att ttc cag aat ttg acc aaa aaa caa aga caa 1680
Glu Glu Asn Cys Asp Ile Phe Gln Asn Leu Thr Lys Lys Gln Arg Gln
545 550 555 560

tct tta agg aaa atg gtc att gac atc gta ctt gca aca gat atg tca 1728
Ser Leu Arg Lys Met Val Ile Asp Ile Val Leu Ala Thr Asp Met Ser
565 570 575

aaa cac atg aat cta ctg gct gat ttg aag act atg gtt gaa act aag 1776
Lys His Met Asn Leu Leu Ala Asp Leu Lys Thr Met Val Glu Thr Lys
580 585 590

aaa gtg aca agc tct gga gtt ctt ctt ctt gat aat tat tcc gat agg 1824
Lys Val Thr Ser Ser Gly Val Leu Leu Leu Asp Asn Tyr Ser Asp Arg
595 600 605

att cag gtt ctt cag aat atg gtg cac tgt gca gat ctg agc aac cca 1872
Ile Gln Val Leu Gln Asn Met Val His Cys Ala Asp Leu Ser Asn Pro
610 615 620

aca aag cct ctc cag ctg tac cgc cag tgg acg gac cgg ata atg gag 1920
Thr Lys Pro Leu Gln Leu Tyr Arg Gln Trp Thr Asp Arg Ile Met Glu
625 630 635 640

gag ttc ttc cgc caa gga gac cga gag agg gaa cgt ggc atg gag ata 1968
Glu Phe Phe Arg Gln Gly Asp Arg Glu Arg Glu Arg Gly Met Glu Ile
645 650 655

agc ccc atg tgt gac aag cac aat gct tcc gtg gaa aaa tca cag gtg 2016
Ser Pro Met Cys Asp Lys His Asn Ala Ser Val Glu Lys Ser Gln Val
660 665 670

ggc ttc ata gac tat att gtt cat ccc ctc tgg gag aca tgg gca gac 2064
Gly Phe Ile Asp Tyr Ile Val His Pro Leu Trp Glu Thr Trp Ala Asp
675 680 685

ctc gtc cac cct gac gcc cag gat att ttg gac act ttg gag gac aat 2112
Leu Val His Pro Asp Ala Gln Asp Ile Leu Asp Thr Leu Glu Asp Asn
690 695 700

cgt gaa tgg tac cag agc aca atc cct cag agc ccc tct cct gca cct 2160
Arg Glu Trp Tyr Gln Ser Thr Ile Pro Gln Ser Pro Ser Pro Ala Pro
705 710 715 720

gat gac cca gag gag ggc cgg cag ggt caa act gag aaa ttc cag ttt 2208
Asp Asp Pro Glu Glu Gly Arg Gln Gly Gln Thr Glu Lys Phe Gln Phe
725 730 735

gaa cta act tta gag gaa gat ggt gag tca gac acg gaa aag gac agt 2256
Glu Leu Thr Leu Glu Glu Asp Gly Glu Ser Asp Thr Glu Lys Asp Ser
740 745 750

ggc agt caa gtg gaa gaa gac act agc tgc agt gac tcc aag act ctt 2304
Gly Ser Gln Val Glu Glu Asp Thr Ser Cys Ser Asp Ser Lys Thr Leu
755 760 765

tgt act caa gac tca gag tct act gaa att ccc ctt gat gaa cag gtt 2352

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Cys Thr Gln Asp Ser Glu Ser Thr Glu Ile Pro Leu Asp Glu Gln Val
    770                 775                 780

gaa gag gag gca gta ggg gaa gaa gag gaa agc cag cct gaa gcc tgt      2400
Glu Glu Glu Ala Val Gly Glu Glu Glu Glu Ser Gln Pro Glu Ala Cys
785                 790                 795                 800

gtc ata gat gat cgt tct cct gac acg acg gga att ctg cag tcg acg      2448
Val Ile Asp Asp Arg Ser Pro Asp Thr Thr Gly Ile Leu Gln Ser Thr
                805                 810                 815

gta ccg cgg gcc cgg gat cca ccg gtc gcc acc atg gtg agc aag ggc      2496
Val Pro Arg Ala Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly
            820                 825                 830

gag gag ctg ttc acc ggg gtg gtg ccc atc ctg gtc gag ctg gac ggc      2544
Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly
        835                 840                 845

gac gta aac ggc cac aag ttc agc gtg tcc ggc gag ggc gag ggc gat      2592
Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp
    850                 855                 860

gcc acc tac ggc aag ctg acc ctg aag ttc atc tgc acc acc ggc aag      2640
Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys
865                 870                 875                 880

ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc ctg acc tac ggc gtg      2688
Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val
                885                 890                 895

cag tgc ttc agc cgc tac ccc gac cac atg aag cag cac gac ttc ttc      2736
Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe
            900                 905                 910

aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc acc atc ttc ttc      2784
Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe
        915                 920                 925

aag gac gac ggc aac tac aag acc cgc gcc gag gtg aag ttc gag ggc      2832
Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly
    930                 935                 940

gac acc ctg gtg aac cgc atc gag ctg aag ggc atc gac ttc aag gag      2880
Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu
945                 950                 955                 960

gac ggc aac atc ctg ggg cac aag ctg gag tac aac tac aac agc cac      2928
Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His
                965                 970                 975

aac gtc tat atc atg gcc gac aag cag aag aac ggc atc aag gtg aac      2976
Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn
            980                 985                 990

ttc aag atc cgc cac aac atc gag gac ggc agc gtg cag ctc gcc gac      3024
Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp
        995                 1000                1005
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cac tac cag cag aac acc ccc atc ggc gac ggc ccc gtg ctg ctg ccc      3072
His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro
    1010                1015                1020

gac aac cac tac ctg agc acc cag tcc gcc ctg agc aaa gac ccc aac      3120
Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn
1025                1030                1035                1040

gag aag cgc gat cac atg gtc ctg ctg gag ttc gtg acc gcc gcc ggg      3168
Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly
                1045                1050                1055

atc act ctc ggc atg gac gag ctg tac aag taa                          3201
Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys  *
            1060                1065


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      <211> 1066
      <212> PRT
      <213> Aequorea victoria and human

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1               5                   10                  15
Gly Ser Asp Ser Ala Gly Gly Ala Thr Leu Lys Ala Pro Lys His Leu
            20                  25                  30
Trp Arg His Glu Gln His His Gln Tyr Pro Leu Arg Gln Pro Gln Phe
        35                  40                  45
Arg Leu Leu His Pro His His His Leu Pro Pro Pro Pro Pro Pro Ser
    50                  55                  60
Pro Gln Pro Gln Pro Gln Cys Pro Leu Gln Pro Pro Pro Pro Pro Pro
65                  70                  75                  80
Leu Pro Pro Pro Pro Pro Pro Pro Gly Ala Ala Arg Gly Arg Tyr Ala
                85                  90                  95
Ser Ser Gly Ala Thr Gly Arg Val Arg His Arg Gly Tyr Ser Asp Thr
            100                 105                 110
Glu Arg Tyr Leu Tyr Cys Arg Ala Met Asp Arg Thr Ser Tyr Ala Val
        115                 120                 125
Glu Thr Gly His Arg Pro Gly Leu Lys Lys Ser Arg Met Ser Trp Pro
    130                 135                 140
Ser Ser Phe Gln Gly Leu Arg Arg Phe Asp Val Asp Asn Gly Thr Ser
145                 150                 155                 160
Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser Gly Leu
                165                 170                 175
Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser Phe Leu
            180                 185                 190
Tyr Arg Ser Asp Ser Asp Tyr Asp Leu Ser Pro Lys Ser Met Ser Arg
        195                 200                 205
Asn Ser Ser Ile Ala Ser Asp Ile His Gly Asp Asp Leu Ile Val Thr
    210                 215                 220
Pro Phe Ala Gln Val Leu Ala Ser Leu Arg Thr Val Arg Asn Asn Phe
225                 230                 235                 240
Ala Ala Leu Thr Asn Leu Gln Asp Arg Ala Pro Ser Lys Arg Ser Pro
                245                 250                 255
Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Glu Glu Ala
            260                 265                 270
Tyr Gln Lys Leu Ala Ser Glu Thr Leu Glu Glu Leu Asp Trp Cys Leu
        275                 280                 285
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Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu Met Ala
290 295 300
Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His Leu Ser
305 310 315 320
Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser Asn Thr
325 330 335
Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr Gln Lys
340 345 350
Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly Val Lys
355 360 365
Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro Arg Phe
370 375 380
Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu Glu Asp
385 390 395 400
Val Asn Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu Ser Gly
405 410 415
Asn Arg Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu Arg Asp
420 425 430
Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr Tyr Leu
435 440 445
Met Thr Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His Asn Asn
450 455 460
Ile His Ala Ala Asp Val Val Gln Ser Thr His Val Leu Leu Ser Thr
465 470 475 480
Pro Ala Leu Glu Ala Val Phe Thr Asp Leu Glu Ile Leu Ala Ala Ile
485 490 495
Phe Ala Ser Ala Ile His Asp Val Asp His Pro Gly Val Ser Asn Gln
500 505 510
Phe Leu Ile Asn Thr Asn Ser Glu Leu Ala Leu Met Tyr Asn Asp Ser
515 520 525
Ser Val Leu Glu Asn His His Leu Ala Val Gly Phe Lys Leu Leu Gln
530 535 540
Glu Glu Asn Cys Asp Ile Phe Gln Asn Leu Thr Lys Lys Gln Arg Gln
545 550 555 560
Ser Leu Arg Lys Met Val Ile Asp Ile Val Leu Ala Thr Asp Met Ser
565 570 575
Lys His Met Asn Leu Leu Ala Asp Leu Lys Thr Met Val Glu Thr Lys
580 585 590
Lys Val Thr Ser Ser Gly Val Leu Leu Leu Asp Asn Tyr Ser Asp Arg
595 600 605
Ile Gln Val Leu Gln Asn Met Val His Cys Ala Asp Leu Ser Asn Pro
610 615 620
Thr Lys Pro Leu Gln Leu Tyr Arg Gln Trp Thr Asp Arg Ile Met Glu
625 630 635 640
Glu Phe Phe Arg Gln Gly Asp Arg Glu Arg Glu Arg Gly Met Glu Ile
645 650 655
Ser Pro Met Cys Asp Lys His Asn Ala Ser Val Glu Lys Ser Gln Val
660 665 670
Gly Phe Ile Asp Tyr Ile Val His Pro Leu Trp Glu Thr Trp Ala Asp
675 680 685
Leu Val His Pro Asp Ala Gln Asp Ile Leu Asp Thr Leu Glu Asp Asn
690 695 700
Arg Glu Trp Tyr Gln Ser Thr Ile Pro Gln Ser Pro Ser Pro Ala Pro
705 710 715 720
Asp Asp Pro Glu Glu Gly Arg Gln Gly Gln Thr Glu Lys Phe Gln Phe
725 730 735
Glu Leu Thr Leu Glu Glu Asp Gly Glu Ser Asp Thr Glu Lys Asp Ser
740 745 750
Gly Ser Gln Val Glu Glu Asp Thr Ser Cys Ser Asp Ser Lys Thr Leu

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        755                 760                 765
Cys Thr Gln Asp Ser Glu Ser Thr Glu Ile Pro Leu Asp Glu Gln Val
    770                 775                 780
Glu Glu Glu Ala Val Gly Glu Glu Glu Glu Ser Gln Pro Glu Ala Cys
785                 790                 795                 800
Val Ile Asp Asp Arg Ser Pro Asp Thr Thr Gly Ile Leu Gln Ser Thr
                805                 810                 815
Val Pro Arg Ala Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly
            820                 825                 830
Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly
        835                 840                 845
Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp
    850                 855                 860
Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys
865                 870                 875                 880
Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val
                885                 890                 895
Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe
            900                 905                 910
Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe
        915                 920                 925
Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly
    930                 935                 940
Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu
945                 950                 955                 960
Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His
                965                 970                 975
Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn
            980                 985                 990
Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp
        995                 1000                1005
His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro
    1010                1015                1020
Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn
1025                1030                1035                1040
Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly
                1045                1050                1055
Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
            1060                1065
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<221> CDS
<222> (1)...(3009)

<400> 5

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Met Ala Gln Gln Thr Ser Pro Asp Thr Leu Thr Val Pro Glu Val Asp
 1               5                  10                  15

aat ccg cat tgt cca aac ccg tgg ctg aac gaa gac ctt gtg aaa tcc      96
Asn Pro His Cys Pro Asn Pro Trp Leu Asn Glu Asp Leu Val Lys Ser
            20                  25                  30

ttg cga gaa aac ctg ttg cag cat gag aag tcc aag aca gcg agg aaa     144
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Leu Arg Glu Asn Leu Leu Gln His Glu Lys Ser Lys Thr Ala Arg Lys
        35                  40                  45

tcg gtt tct ccc aag ctc tct cca gtg atc tct ccg aga aat tcc ccc       192
Ser Val Ser Pro Lys Leu Ser Pro Val Ile Ser Pro Arg Asn Ser Pro
    50                  55                  60

agg ctt ctg cgc aga atg ctt ctc agc agc aac atc ccc aaa cag cgg       240
Arg Leu Leu Arg Arg Met Leu Leu Ser Ser Asn Ile Pro Lys Gln Arg
 65                 70                  75                  80

cgt ttc acg gtg gca cat aca tgt ttt gat gtg gac aat ggc aca tct       288
Arg Phe Thr Val Ala His Thr Cys Phe Asp Val Asp Asn Gly Thr Ser
                85                  90                  95

gcg gga cgg agt ccc ttg gat ccc atg acc agc cca gga tcc ggg cta       336
Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser Gly Leu
            100                 105                 110

att ctc caa gca aat ttt gtc cac agt caa cga cgg gag tcc ttc ctg       384
Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser Phe Leu
        115                 120                 125

tat cga tcc gac agc gat tat gac ctc tct cca aag tct atg tcc cgg       432
Tyr Arg Ser Asp Ser Asp Tyr Asp Leu Ser Pro Lys Ser Met Ser Arg
    130                 135                 140

aac tcc tcc att gcc agt gat ata cac gga gat gac ttg att gtg act       480
Asn Ser Ser Ile Ala Ser Asp Ile His Gly Asp Asp Leu Ile Val Thr
145                 150                 155                 160

cca ttt gct cag gtc ttg gcc agt ctg cga act gta cga aac aac ttt       528
Pro Phe Ala Gln Val Leu Ala Ser Leu Arg Thr Val Arg Asn Asn Phe
                165                 170                 175

gct gca tta act aat ttg caa gat cga gca cct agc aaa aga tca ccc       576
Ala Ala Leu Thr Asn Leu Gln Asp Arg Ala Pro Ser Lys Arg Ser Pro
            180                 185                 190

atg tgc aac caa cca tcc atc aac aaa gcc acc ata aca gag gag gcc       624
Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Glu Glu Ala
        195                 200                 205

tac cag aaa ctg gcc agc gag acc ctg gag gag ctg gac tgg tgt ctg       672
Tyr Gln Lys Leu Ala Ser Glu Thr Leu Glu Glu Leu Asp Trp Cys Leu
    210                 215                 220

gac cag cta gag acc cta cag acc agg cac tcc gtc agt gag atg gcc       720
Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu Met Ala
225                 230                 235                 240

tcc aac aag ttt aaa agg atg ctt aat cgg gag ctc acc cat ctc tct       768
Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His Leu Ser
                245                 250                 255

gaa atg agt cgg tct gga aat caa gtg tca gag ttt ata tca aac aca       816
Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser Asn Thr
            260                 265                 270
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ttc tta gat aag caa cat gaa gtg gaa att cct tct cca act cag aag        864
Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr Gln Lys
        275                 280                 285

gaa aag gag aaa aag aaa aga cca atg tct cag atc agt gga gtc aag        912
Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly Val Lys
    290                 295                 300

aaa ttg atg cac agc tct agt ctg act aat tca agt atc cca agg ttt        960
Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro Arg Phe
305                 310                 315                 320

gga gtt aaa act gaa caa gaa gat gtc ctt gcc aag gaa cta gaa gat       1008
Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu Glu Asp
                325                 330                 335

gtg aac aaa tgg ggt ctt cat gtt ttc aga ata gca gag ttg tct ggt       1056
Val Asn Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu Ser Gly
            340                 345                 350

aac cgg ccc ttg act gtt atc atg cac acc att ttt cag gaa cgg gat       1104
Asn Arg Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu Arg Asp
        355                 360                 365

tta tta aaa aca ttt aaa att cca gta gat act tta att aca tat ctt       1152
Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr Tyr Leu
    370                 375                 380

atg act ctc gaa gac cat tac cat gct gat gtg gcc tat cac aac aat       1200
Met Thr Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His Asn Asn
385                 390                 395                 400

atc cat gct gca gat gtt gtc cag tct act cat gtg cta tta tct aca       1248
Ile His Ala Ala Asp Val Val Gln Ser Thr His Val Leu Leu Ser Thr
                405                 410                 415

cct gct ttg gag gct gtg ttt aca gat ttg gag att ctt gca gca att       1296
Pro Ala Leu Glu Ala Val Phe Thr Asp Leu Glu Ile Leu Ala Ala Ile
            420                 425                 430

ttt gcc agt gca ata cat gat gta gat cat cct ggt gtg tcc aat caa       1344
Phe Ala Ser Ala Ile His Asp Val Asp His Pro Gly Val Ser Asn Gln
        435                 440                 445

ttt ctg atc aat aca aac tct gaa ctt gcc ttg atg tac aat gat tcc       1392
Phe Leu Ile Asn Thr Asn Ser Glu Leu Ala Leu Met Tyr Asn Asp Ser
    450                 455                 460

tca gtc tta gag aac cat cat ttg gct gtg ggc ttt aaa ttg ctt cag       1440
Ser Val Leu Glu Asn His His Leu Ala Val Gly Phe Lys Leu Leu Gln
465                 470                 475                 480

gaa gaa aac tgt gac att ttc cag aat ttg acc aaa aaa caa aga caa       1488
Glu Glu Asn Cys Asp Ile Phe Gln Asn Leu Thr Lys Lys Gln Arg Gln
                485                 490                 495

tct tta agg aaa atg gtc att gac atc gta ctt gca aca gat atg tca       1536
Ser Leu Arg Lys Met Val Ile Asp Ile Val Leu Ala Thr Asp Met Ser
            500                 505                 510
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aaa cac atg aat cta ctg gct gat ttg aag act atg gtt gaa act aag      1584
Lys His Met Asn Leu Leu Ala Asp Leu Lys Thr Met Val Glu Thr Lys
        515                 520                 525

aaa gtg aca agc tct gga gtt ctt ctt ctt gat aat tat tcc gat agg      1632
Lys Val Thr Ser Ser Gly Val Leu Leu Leu Asp Asn Tyr Ser Asp Arg
    530                 535                 540

att cag gtt ctt cag aat atg gtg cac tgt gca gat ctg agc aac cca      1680
Ile Gln Val Leu Gln Asn Met Val His Cys Ala Asp Leu Ser Asn Pro
545                 550                 555                 560

aca aag cct ctc cag ctg tac cgc cag tgg acg gac cgg ata atg gag      1728
Thr Lys Pro Leu Gln Leu Tyr Arg Gln Trp Thr Asp Arg Ile Met Glu
                565                 570                 575

gag ttc ttc cgc caa gga gac cga gag agg gaa cgt ggc atg gag ata      1776
Glu Phe Phe Arg Gln Gly Asp Arg Glu Arg Glu Arg Gly Met Glu Ile
            580                 585                 590

agc ccc atg tgt gac aag cac aat gct tcc gtg gaa aaa tca cag gtg      1824
Ser Pro Met Cys Asp Lys His Asn Ala Ser Val Glu Lys Ser Gln Val
        595                 600                 605

ggc ttc ata gac tat att gtt cat ccc ctc tgg gag aca tgg gca gac      1872
Gly Phe Ile Asp Tyr Ile Val His Pro Leu Trp Glu Thr Trp Ala Asp
    610                 615                 620

ctc gtc cac cct gac gcc cag gat att ttg gac act ttg gag gac aat      1920
Leu Val His Pro Asp Ala Gln Asp Ile Leu Asp Thr Leu Glu Asp Asn
625                 630                 635                 640

cgt gaa tgg tac cag agc aca atc cct cag agc ccc tct cct gca cct      1968
Arg Glu Trp Tyr Gln Ser Thr Ile Pro Gln Ser Pro Ser Pro Ala Pro
                645                 650                 655

gat gac cca gag gag ggc cgg cag ggt caa act gag aaa ttc cag ttt      2016
Asp Asp Pro Glu Glu Gly Arg Gln Gly Gln Thr Glu Lys Phe Gln Phe
            660                 665                 670

gaa cta act tta gag gaa gat ggt gag tca gac acg gaa aag gac agt      2064
Glu Leu Thr Leu Glu Glu Asp Gly Glu Ser Asp Thr Glu Lys Asp Ser
        675                 680                 685

ggc agt caa gtg gaa gaa gac act agc tgc agt gac tcc aag act ctt      2112
Gly Ser Gln Val Glu Glu Asp Thr Ser Cys Ser Asp Ser Lys Thr Leu
    690                 695                 700

tgt act caa gac tca gag tct act gaa att ccc ctt gat gaa cag gtt      2160
Cys Thr Gln Asp Ser Glu Ser Thr Glu Ile Pro Leu Asp Glu Gln Val
705                 710                 715                 720

gaa gag gag gca gta ggg gaa gaa gag gaa agc cag cct gaa gcc tgt      2208
Glu Glu Glu Ala Val Gly Glu Glu Glu Glu Ser Gln Pro Glu Ala Cys
                725                 730                 735

gtc ata gat gat cgt tct cct gac acg acg gga att ctg cag tcg acg      2256
Val Ile Asp Asp Arg Ser Pro Asp Thr Thr Gly Ile Leu Gln Ser Thr
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                740                 745                 750

gta ccg cgg gcc cgg gat cca ccg gtc gcc acc atg gtg agc aag ggc       2304
Val Pro Arg Ala Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly
            755                 760                 765

gag gag ctg ttc acc ggg gtg gtg ccc atc ctg gtc gag ctg gac ggc       2352
Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly
        770                 775                 780

gac gta aac ggc cac aag ttc agc gtg tcc ggc gag ggc gag ggc gat       2400
Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp
785                 790                 795                 800

gcc acc tac ggc aag ctg acc ctg aag ttc atc tgc acc acc ggc aag       2448
Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys
                805                 810                 815

ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc ctg acc tac ggc gtg       2496
Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val
            820                 825                 830

cag tgc ttc agc cgc tac ccc gac cac atg aag cag cac gac ttc ttc       2544
Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe
        835                 840                 845

aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc acc atc ttc ttc       2592
Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe
    850                 855                 860

aag gac gac ggc aac tac aag acc cgc gcc gag gtg aag ttc gag ggc       2640
Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly
865                 870                 875                 880

gac acc ctg gtg aac cgc atc gag ctg aag ggc atc gac ttc aag gag       2688
Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu
                885                 890                 895

gac ggc aac atc ctg ggg cac aag ctg gag tac aac tac aac agc cac       2736
Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His
            900                 905                 910

aac gtc tat atc atg gcc gac aag cag aag aac ggc atc aag gtg aac       2784
Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn
        915                 920                 925

ttc aag atc cgc cac aac atc gag gac ggc agc gtg cag ctc gcc gac       2832
Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp
    930                 935                 940

cac tac cag cag aac acc ccc atc ggc gac ggc ccc gtg ctg ctg ccc       2880
His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro
945                 950                 955                 960

gac aac cac tac ctg agc acc cag tcc gcc ctg agc aaa gac ccc aac       2928
Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn
                965                 970                 975

gag aag cgc gat cac atg gtc ctg ctg gag ttc gtg acc gcc gcc ggg       2976
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Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly
980 985 990

atc act ctc ggc atg gac gag ctg tac aag taa 3009
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995 1000

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35 40 45
Ser Val Ser Pro Lys Leu Ser Pro Val Ile Ser Pro Arg Asn Ser Pro
50 55 60
Arg Leu Leu Arg Arg Met Leu Leu Ser Ser Asn Ile Pro Lys Gln Arg
65 70 75 80
Arg Phe Thr Val Ala His Thr Cys Phe Asp Val Asp Asn Gly Thr Ser
85 90 95
Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser Gly Leu
100 105 110
Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser Phe Leu
115 120 125
Tyr Arg Ser Asp Ser Asp Tyr Asp Leu Ser Pro Lys Ser Met Ser Arg
130 135 140
Asn Ser Ser Ile Ala Ser Asp Ile His Gly Asp Asp Leu Ile Val Thr
145 150 155 160
Pro Phe Ala Gln Val Leu Ala Ser Leu Arg Thr Val Arg Asn Asn Phe
165 170 175
Ala Ala Leu Thr Asn Leu Gln Asp Arg Ala Pro Ser Lys Arg Ser Pro
180 185 190
Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Glu Glu Ala
195 200 205
Tyr Gln Lys Leu Ala Ser Glu Thr Leu Glu Glu Leu Asp Trp Cys Leu
210 215 220
Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu Met Ala
225 230 235 240
Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His Leu Ser
245 250 255
Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser Asn Thr
260 265 270
Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr Gln Lys
275 280 285
Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly Val Lys
290 295 300
Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro Arg Phe
305 310 315 320
Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu Glu Asp
325 330 335
Val Asn Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu Ser Gly
340 345 350
Asn Arg Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu Arg Asp

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        355                 360                 365
Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr Tyr Leu
    370                 375                 380
Met Thr Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His Asn Asn
385                 390                 395                 400
Ile His Ala Ala Asp Val Val Gln Ser Thr His Val Leu Leu Ser Thr
                405                 410                 415
Pro Ala Leu Glu Ala Val Phe Thr Asp Leu Glu Ile Leu Ala Ala Ile
            420                 425                 430
Phe Ala Ser Ala Ile His Asp Val Asp His Pro Gly Val Ser Asn Gln
        435                 440                 445
Phe Leu Ile Asn Thr Asn Ser Glu Leu Ala Leu Met Tyr Asn Asp Ser
    450                 455                 460
Ser Val Leu Glu Asn His His Leu Ala Val Gly Phe Lys Leu Leu Gln
465                 470                 475                 480
Glu Glu Asn Cys Asp Ile Phe Gln Asn Leu Thr Lys Lys Gln Arg Gln
                485                 490                 495
Ser Leu Arg Lys Met Val Ile Asp Ile Val Leu Ala Thr Asp Met Ser
            500                 505                 510
Lys His Met Asn Leu Leu Ala Asp Leu Lys Thr Met Val Glu Thr Lys
        515                 520                 525
Lys Val Thr Ser Ser Gly Val Leu Leu Leu Asp Asn Tyr Ser Asp Arg
    530                 535                 540
Ile Gln Val Leu Gln Asn Met Val His Cys Ala Asp Leu Ser Asn Pro
545                 550                 555                 560
Thr Lys Pro Leu Gln Leu Tyr Arg Gln Trp Thr Asp Arg Ile Met Glu
                565                 570                 575
Glu Phe Phe Arg Gln Gly Asp Arg Glu Arg Glu Arg Gly Met Glu Ile
            580                 585                 590
Ser Pro Met Cys Asp Lys His Asn Ala Ser Val Glu Lys Ser Gln Val
        595                 600                 605
Gly Phe Ile Asp Tyr Ile Val His Pro Leu Trp Glu Thr Trp Ala Asp
    610                 615                 620
Leu Val His Pro Asp Ala Gln Asp Ile Leu Asp Thr Leu Glu Asp Asn
625                 630                 635                 640
Arg Glu Trp Tyr Gln Ser Thr Ile Pro Gln Ser Pro Ser Pro Ala Pro
                645                 650                 655
Asp Asp Pro Glu Glu Gly Arg Gln Gly Gln Thr Glu Lys Phe Gln Phe
            660                 665                 670
Glu Leu Thr Leu Glu Glu Asp Gly Glu Ser Asp Thr Glu Lys Asp Ser
        675                 680                 685
Gly Ser Gln Val Glu Glu Asp Thr Ser Cys Ser Asp Ser Lys Thr Leu
    690                 695                 700
Cys Thr Gln Asp Ser Glu Ser Thr Glu Ile Pro Leu Asp Glu Gln Val
705                 710                 715                 720
Glu Glu Glu Ala Val Gly Glu Glu Glu Glu Ser Gln Pro Glu Ala Cys
                725                 730                 735
Val Ile Asp Asp Arg Ser Pro Asp Thr Thr Gly Ile Leu Gln Ser Thr
            740                 745                 750
Val Pro Arg Ala Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly
        755                 760                 765
Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly
    770                 775                 780
Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp
785                 790                 795                 800
Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys
                805                 810                 815
Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val
            820                 825                 830
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Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe
835 840 845
Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe
850 855 860
Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly
865 870 875 880
Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu
885 890 895
Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His
900 905 910
Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn
915 920 925
Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp
930 935 940
His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro
945 950 955 960
Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn
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980 985 990
Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
995 1000

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ccc cag cag cag aag cag cag cag agg gat cag gac tcg gtc gaa gca 96
Pro Gln Gln Gln Lys Gln Gln Gln Arg Asp Gln Asp Ser Val Glu Ala
20 25 30

tgg ctg gac gat cac tgg gac ttt acc ttc tca tac ttt gtt aga aaa 144
Trp Leu Asp Asp His Trp Asp Phe Thr Phe Ser Tyr Phe Val Arg Lys
35 40 45

gcc acc aga gaa atg gtc aat gca tgg ttt gct gag aga gtt cac acc 192
Ala Thr Arg Glu Met Val Asn Ala Trp Phe Ala Glu Arg Val His Thr
50 55 60

atc cct gtg tgc aag gaa ggt atc aga ggc cac acc gaa tct tgc tct 240
Ile Pro Val Cys Lys Glu Gly Ile Arg Gly His Thr Glu Ser Cys Ser
65 70 75 80

tgt ccc ttg cag cag agt cct cgt gca gat aac agt gtc cct gga aca 288
Cys Pro Leu Gln Gln Ser Pro Arg Ala Asp Asn Ser Val Pro Gly Thr
85 90 95

cca acc agg aaa atc tct gcc tct gaa ttt gac cgg cct ctt aga ccc 336
Pro Thr Arg Lys Ile Ser Ala Ser Glu Phe Asp Arg Pro Leu Arg Pro

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            100                 105                 110

att gtt gtc aag gat tct gag gga act gtg agc ttc ctc tct gac tca      384
Ile Val Val Lys Asp Ser Glu Gly Thr Val Ser Phe Leu Ser Asp Ser
        115                 120                 125

gaa aag aag gaa cag atg cct cta acc cct cca agg ttt gat cat gat      432
Glu Lys Lys Glu Gln Met Pro Leu Thr Pro Pro Arg Phe Asp His Asp
    130                 135                 140

gaa ggg gac cag tgc tca aga ctc ttg gaa tta gtg aag gat att tct      480
Glu Gly Asp Gln Cys Ser Arg Leu Leu Glu Leu Val Lys Asp Ile Ser
145                 150                 155                 160

agt cat ttg gat gtc aca gcc tta tgt cac aaa att ttc ttg cat atc      528
Ser His Leu Asp Val Thr Ala Leu Cys His Lys Ile Phe Leu His Ile
                165                 170                 175

cat gga ctg ata tct gct gac cgc tat tcc ctg ttc ctt gtc tgt gaa      576
His Gly Leu Ile Ser Ala Asp Arg Tyr Ser Leu Phe Leu Val Cys Glu
            180                 185                 190

gac agc tcc aat gac aag ttt ctt atc agc cgc ctc ttt gat gtt gct      624
Asp Ser Ser Asn Asp Lys Phe Leu Ile Ser Arg Leu Phe Asp Val Ala
        195                 200                 205

gaa ggt tca aca ctg gaa gaa gtt tca aat aac tgt atc cgc tta gaa      672
Glu Gly Ser Thr Leu Glu Glu Val Ser Asn Asn Cys Ile Arg Leu Glu
    210                 215                 220

tgg aac aaa ggc att gtg gga cat gtg gca gcg ctt ggt gag ccc ttg      720
Trp Asn Lys Gly Ile Val Gly His Val Ala Ala Leu Gly Glu Pro Leu
225                 230                 235                 240

aac atc aaa gat gca tat gag gat cct cgg ttc aat gca gaa gtt gac      768
Asn Ile Lys Asp Ala Tyr Glu Asp Pro Arg Phe Asn Ala Glu Val Asp
                245                 250                 255

caa att aca ggc tac aag aca caa agc att ctt tgt atg cca att aag      816
Gln Ile Thr Gly Tyr Lys Thr Gln Ser Ile Leu Cys Met Pro Ile Lys
            260                 265                 270

aat cat agg gaa gag gtt gtt ggt gta gcc cag gcc atc aac aag aaa      864
Asn His Arg Glu Glu Val Val Gly Val Ala Gln Ala Ile Asn Lys Lys
        275                 280                 285

tca gga aac ggt ggg aca ttt act gaa aaa gat gaa aag gac ttt gct      912
Ser Gly Asn Gly Gly Thr Phe Thr Glu Lys Asp Glu Lys Asp Phe Ala
    290                 295                 300

gct tat ttg gca ttt tgt ggt att gtt ctt cat aat gct cag ctc tat      960
Ala Tyr Leu Ala Phe Cys Gly Ile Val Leu His Asn Ala Gln Leu Tyr
305                 310                 315                 320

gag act tca ctg ctg gag aac aag aga aat cag gtg ctg ctt gac ctt     1008
Glu Thr Ser Leu Leu Glu Asn Lys Arg Asn Gln Val Leu Leu Asp Leu
                325                 330                 335

gct agt tta att ttt gaa gaa caa caa tca tta gaa gta att ttg aag     1056
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Ala Ser Leu Ile Phe Glu Glu Gln Gln Ser Leu Glu Val Ile Leu Lys
340 345 350

aaa ata gct gcc act att atc tct ttc atg caa gtg cag aaa tgc acc 1104
Lys Ile Ala Ala Thr Ile Ile Ser Phe Met Gln Val Gln Lys Cys Thr
355 360 365

att ttc ata gtg gat gaa gat tgc tcc gat tct ttt tct agt gtg ttt 1152
Ile Phe Ile Val Asp Glu Asp Cys Ser Asp Ser Phe Ser Ser Val Phe
370 375 380

cac atg gag tgt gag gaa tta gaa aaa tca tct gat aca tta aca agg 1200
His Met Glu Cys Glu Glu Leu Glu Lys Ser Ser Asp Thr Leu Thr Arg
385 390 395 400

gaa cat gat gca aac aaa atc aat tac atg tat gct cag tat gtc aaa 1248
Glu His Asp Ala Asn Lys Ile Asn Tyr Met Tyr Ala Gln Tyr Val Lys
405 410 415

aat act atg gaa cca ctt aat atc cca gat gtc agt aag gat aaa aga 1296
Asn Thr Met Glu Pro Leu Asn Ile Pro Asp Val Ser Lys Asp Lys Arg
420 425 430

ttt ccc tgg aca act gaa aat aca gga aat gta aac cag cag tgc att 1344
Phe Pro Trp Thr Thr Glu Asn Thr Gly Asn Val Asn Gln Gln Cys Ile
435 440 445

aga agt ttg ctt tgt aca cct ata aaa aat gga aag aag aat aaa gtt 1392
Arg Ser Leu Leu Cys Thr Pro Ile Lys Asn Gly Lys Lys Asn Lys Val
450 455 460

ata ggg gtt tgc caa ctt gtt aat aag atg gag gag aat act ggc aag 1440
Ile Gly Val Cys Gln Leu Val Asn Lys Met Glu Glu Asn Thr Gly Lys
465 470 475 480

gtt aag cct ttc aac cga aat gac gaa cag ttt ctg gaa gct ttt gtc 1488
Val Lys Pro Phe Asn Arg Asn Asp Glu Gln Phe Leu Glu Ala Phe Val
485 490 495

atc ttt tgt ggc ttg ggg atc cag aac acg cag atg tat gaa gca gtg 1536
Ile Phe Cys Gly Leu Gly Ile Gln Asn Thr Gln Met Tyr Glu Ala Val
500 505 510

gag aga gcc atg gcc aag caa atg gtc aca ttg gag gtt ctg tcg tat 1584
Glu Arg Ala Met Ala Lys Gln Met Val Thr Leu Glu Val Leu Ser Tyr
515 520 525

cat gct tca gca gca gag gaa gaa aca aga gag cta cag tcg tta gcg 1632
His Ala Ser Ala Ala Glu Glu Glu Thr Arg Glu Leu Gln Ser Leu Ala
530 535 540

gct gct gtg gtg cca tct gcc cag acc ctt aaa att act gac ttt agc 1680
Ala Ala Val Val Pro Ser Ala Gln Thr Leu Lys Ile Thr Asp Phe Ser
545 550 555 560

ttc agt gac ttt gag ctg tct gat ctg gaa aca gca ctg tgc aca att 1728
Phe Ser Asp Phe Glu Leu Ser Asp Leu Glu Thr Ala Leu Cys Thr Ile
565 570 575

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cgg atg ttt act gac ctc aac ctt gtg cag aac ttc cag atg aaa cat      1776
Arg Met Phe Thr Asp Leu Asn Leu Val Gln Asn Phe Gln Met Lys His
            580                 585                 590

gag gtt ctt tgc aga tgg att tta agt gtt aag aag aat tat cgg aag      1824
Glu Val Leu Cys Arg Trp Ile Leu Ser Val Lys Lys Asn Tyr Arg Lys
        595                 600                 605

aat gtt gcc tat cat aat tgg aga cat gcc ttt aat aca gct cag tgc      1872
Asn Val Ala Tyr His Asn Trp Arg His Ala Phe Asn Thr Ala Gln Cys
    610                 615                 620

atg ttt gct gct cta aaa gca ggc aaa att cag aac aag ctg act gac      1920
Met Phe Ala Ala Leu Lys Ala Gly Lys Ile Gln Asn Lys Leu Thr Asp
625                 630                 635                 640

ctg gag ata ctt gca ttg ctg att gct gca cta agc cac gat ttg gat      1968
Leu Glu Ile Leu Ala Leu Leu Ile Ala Ala Leu Ser His Asp Leu Asp
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cac cgt ggt gtg aat aac tct tac ata cag cga agt gaa cat cca ctt      2016
His Arg Gly Val Asn Asn Ser Tyr Ile Gln Arg Ser Glu His Pro Leu
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gcc cag ctt tac tgc cat tca atc atg gaa cac cat cat ttt gac cag      2064
Ala Gln Leu Tyr Cys His Ser Ile Met Glu His His His Phe Asp Gln
        675                 680                 685

tgc ctg atg att ctt aat agt cca ggc aat cag att ctc agt ggc ctc      2112
Cys Leu Met Ile Leu Asn Ser Pro Gly Asn Gln Ile Leu Ser Gly Leu
    690                 695                 700

tcc att gaa gaa tat aag acc acg ttg aaa ata atc aag caa gct att      2160
Ser Ile Glu Glu Tyr Lys Thr Thr Leu Lys Ile Ile Lys Gln Ala Ile
705                 710                 715                 720

tta gct aca gac cta gca ctg tac att aag agg cga gga gaa ttt ttt      2208
Leu Ala Thr Asp Leu Ala Leu Tyr Ile Lys Arg Arg Gly Glu Phe Phe
                725                 730                 735

gaa ctt ata aga aaa aat caa ttc aat ttg gaa gat cct cat caa aag      2256
Glu Leu Ile Arg Lys Asn Gln Phe Asn Leu Glu Asp Pro His Gln Lys
            740                 745                 750

gag ttg ttt ttg gca atg ctg atg aca gct tgt gat ctt tct gca att      2304
Glu Leu Phe Leu Ala Met Leu Met Thr Ala Cys Asp Leu Ser Ala Ile
        755                 760                 765

aca aaa ccc tgg cct att caa caa cgg ata gca gaa ctt gta gca act      2352
Thr Lys Pro Trp Pro Ile Gln Gln Arg Ile Ala Glu Leu Val Ala Thr
    770                 775                 780

gaa ttt ttt gat caa gga gac aga gag aga aaa gaa ctc aac ata gaa      2400
Glu Phe Phe Asp Gln Gly Asp Arg Glu Arg Lys Glu Leu Asn Ile Glu
785                 790                 795                 800

ccc act gat cta atg aac agg gag aag aaa aac aaa atc cca agt atg      2448
Pro Thr Asp Leu Met Asn Arg Glu Lys Lys Asn Lys Ile Pro Ser Met
                805                 810                 815
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caa gtt ggg ttc ata gat gcc atc tgc ttg caa ctg tat gag gcc ctg 2496
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acc cac gtg tca gag gac tgt ttc cct ttg cta gat ggc tgc aga aag 2544
Thr His Val Ser Glu Asp Cys Phe Pro Leu Leu Asp Gly Cys Arg Lys
835 840 845

aac agg cag aaa tgg cag gcc ctt gca gaa cag cag gag aag atg ctg 2592
Asn Arg Gln Lys Trp Gln Ala Leu Ala Glu Gln Gln Glu Lys Met Leu
850 855 860

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Ile Asn Gly Glu Ser Gly Gln Ala Lys Arg Asn Trp Val Pro Arg Ala
865 870 875 880

cgg gat cca ccg gtc gcc acc atg gtg agc aag ggc gag gag ctg ttc 2688
Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly Glu Glu Leu Phe
885 890 895

acc ggg gtg gtg ccc atc ctg gtc gag ctg gac ggc gac gta aac ggc 2736
Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly
900 905 910

cac aag ttc agc gtg tcc ggc gag ggc gag ggc gat gcc acc tac ggc 2784
His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly
915 920 925

aag ctg acc ctg aag ttc atc tgc acc acc ggc aag ctg ccc gtg ccc 2832
Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro
930 935 940

tgg ccc acc ctc gtg acc acc ctg acc tac ggc gtg cag tgc ttc agc 2880
Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser
945 950 955 960

cgc tac ccc gac cac atg aag cag cac gac ttc ttc aag tcc gcc atg 2928
Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met
965 970 975

ccc gaa ggc tac gtc cag gag cgc acc atc ttc ttc aag gac gac ggc 2976
Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly
980 985 990

aac tac aag acc cgc gcc gag gtg aag ttc gag ggc gac acc ctg gtg 3024
Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val
995 1000 1005

aac cgc atc gag ctg aag ggc atc gac ttc aag gag gac ggc aac atc 3072
Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile
1010 1015 1020

ctg ggg cac aag ctg gag tac aac tac aac agc cac aac gtc tat atc 3120
Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile
1025 1030 1035 1040

atg gcc gac aag cag aag aac ggc atc aag gtg aac ttc aag atc cgc 3168
Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg

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                1045                1050                1055

cac aac atc gag gac ggc agc gtg cag ctc gcc gac cac tac cag cag     3216
His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln
            1060                1065                1070

aac acc ccc atc ggc gac ggc ccc gtg ctg ctg ccc gac aac cac tac     3264
Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr
        1075                1080                1085

ctg agc acc cag tcc gcc ctg agc aaa gac ccc aac gag aag cgc gat     3312
Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp
    1090                1095                1100

cac atg gtc ctg ctg gag ttc gtg acc gcc gcc ggg atc act ctc ggc     3360
His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly
1105                1110                1115                1120

atg gac gag ctg tac aag taa                                         3381
Met Asp Glu Leu Tyr Lys  *
                1125


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Trp Leu Asp Asp His Trp Asp Phe Thr Phe Ser Tyr Phe Val Arg Lys
        35                  40                  45
Ala Thr Arg Glu Met Val Asn Ala Trp Phe Ala Glu Arg Val His Thr
    50                  55                  60
Ile Pro Val Cys Lys Glu Gly Ile Arg Gly His Thr Glu Ser Cys Ser
65                  70                  75                  80
Cys Pro Leu Gln Gln Ser Pro Arg Ala Asp Asn Ser Val Pro Gly Thr
                85                  90                  95
Pro Thr Arg Lys Ile Ser Ala Ser Glu Phe Asp Arg Pro Leu Arg Pro
            100                 105                 110
Ile Val Val Lys Asp Ser Glu Gly Thr Val Ser Phe Leu Ser Asp Ser
        115                 120                 125
Glu Lys Lys Glu Gln Met Pro Leu Thr Pro Pro Arg Phe Asp His Asp
    130                 135                 140
Glu Gly Asp Gln Cys Ser Arg Leu Leu Glu Leu Val Lys Asp Ile Ser
145                 150                 155                 160
Ser His Leu Asp Val Thr Ala Leu Cys His Lys Ile Phe Leu His Ile
                165                 170                 175
His Gly Leu Ile Ser Ala Asp Arg Tyr Ser Leu Phe Leu Val Cys Glu
            180                 185                 190
Asp Ser Ser Asn Asp Lys Phe Leu Ile Ser Arg Leu Phe Asp Val Ala
        195                 200                 205
Glu Gly Ser Thr Leu Glu Glu Val Ser Asn Asn Cys Ile Arg Leu Glu
    210                 215                 220
Trp Asn Lys Gly Ile Val Gly His Val Ala Ala Leu Gly Glu Pro Leu
225                 230                 235                 240
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Asn Ile Lys Asp Ala Tyr Glu Asp Pro Arg Phe Asn Ala Glu Val Asp
                245                 250                 255
Gln Ile Thr Gly Tyr Lys Thr Gln Ser Ile Leu Cys Met Pro Ile Lys
            260                 265                 270
Asn His Arg Glu Glu Val Val Gly Val Ala Gln Ala Ile Asn Lys Lys
        275                 280                 285
Ser Gly Asn Gly Gly Thr Phe Thr Glu Lys Asp Glu Lys Asp Phe Ala
    290                 295                 300
Ala Tyr Leu Ala Phe Cys Gly Ile Val Leu His Asn Ala Gln Leu Tyr
305                 310                 315                 320
Glu Thr Ser Leu Leu Glu Asn Lys Arg Asn Gln Val Leu Leu Asp Leu
                325                 330                 335
Ala Ser Leu Ile Phe Glu Glu Gln Gln Ser Leu Glu Val Ile Leu Lys
            340                 345                 350
Lys Ile Ala Ala Thr Ile Ile Ser Phe Met Gln Val Gln Lys Cys Thr
        355                 360                 365
Ile Phe Ile Val Asp Glu Asp Cys Ser Asp Ser Phe Ser Ser Val Phe
    370                 375                 380
His Met Glu Cys Glu Glu Leu Glu Lys Ser Ser Asp Thr Leu Thr Arg
385                 390                 395                 400
Glu His Asp Ala Asn Lys Ile Asn Tyr Met Tyr Ala Gln Tyr Val Lys
                405                 410                 415
Asn Thr Met Glu Pro Leu Asn Ile Pro Asp Val Ser Lys Asp Lys Arg
            420                 425                 430
Phe Pro Trp Thr Thr Glu Asn Thr Gly Asn Val Asn Gln Gln Cys Ile
        435                 440                 445
Arg Ser Leu Leu Cys Thr Pro Ile Lys Asn Gly Lys Lys Asn Lys Val
    450                 455                 460
Ile Gly Val Cys Gln Leu Val Asn Lys Met Glu Glu Asn Thr Gly Lys
465                 470                 475                 480
Val Lys Pro Phe Asn Arg Asn Asp Glu Gln Phe Leu Glu Ala Phe Val
                485                 490                 495
Ile Phe Cys Gly Leu Gly Ile Gln Asn Thr Gln Met Tyr Glu Ala Val
            500                 505                 510
Glu Arg Ala Met Ala Lys Gln Met Val Thr Leu Glu Val Leu Ser Tyr
        515                 520                 525
His Ala Ser Ala Ala Glu Glu Glu Thr Arg Glu Leu Gln Ser Leu Ala
    530                 535                 540
Ala Ala Val Val Pro Ser Ala Gln Thr Leu Lys Ile Thr Asp Phe Ser
545                 550                 555                 560
Phe Ser Asp Phe Glu Leu Ser Asp Leu Glu Thr Ala Leu Cys Thr Ile
                565                 570                 575
Arg Met Phe Thr Asp Leu Asn Leu Val Gln Asn Phe Gln Met Lys His
            580                 585                 590
Glu Val Leu Cys Arg Trp Ile Leu Ser Val Lys Lys Asn Tyr Arg Lys
        595                 600                 605
Asn Val Ala Tyr His Asn Trp Arg His Ala Phe Asn Thr Ala Gln Cys
    610                 615                 620
Met Phe Ala Ala Leu Lys Ala Gly Lys Ile Gln Asn Lys Leu Thr Asp
625                 630                 635                 640
Leu Glu Ile Leu Ala Leu Leu Ile Ala Ala Leu Ser His Asp Leu Asp
                645                 650                 655
His Arg Gly Val Asn Asn Ser Tyr Ile Gln Arg Ser Glu His Pro Leu
            660                 665                 670
Ala Gln Leu Tyr Cys His Ser Ile Met Glu His His His Phe Asp Gln
        675                 680                 685
Cys Leu Met Ile Leu Asn Ser Pro Gly Asn Gln Ile Leu Ser Gly Leu
    690                 695                 700
Ser Ile Glu Glu Tyr Lys Thr Thr Leu Lys Ile Ile Lys Gln Ala Ile
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705                 710                 715                 720
Leu Ala Thr Asp Leu Ala Leu Tyr Ile Lys Arg Arg Gly Glu Phe Phe
                725                 730                 735
Glu Leu Ile Arg Lys Asn Gln Phe Asn Leu Glu Asp Pro His Gln Lys
            740                 745                 750
Glu Leu Phe Leu Ala Met Leu Met Thr Ala Cys Asp Leu Ser Ala Ile
        755                 760                 765
Thr Lys Pro Trp Pro Ile Gln Gln Arg Ile Ala Glu Leu Val Ala Thr
    770                 775                 780
Glu Phe Phe Asp Gln Gly Asp Arg Glu Arg Lys Glu Leu Asn Ile Glu
785                 790                 795                 800
Pro Thr Asp Leu Met Asn Arg Glu Lys Lys Asn Lys Ile Pro Ser Met
                805                 810                 815
Gln Val Gly Phe Ile Asp Ala Ile Cys Leu Gln Leu Tyr Glu Ala Leu
            820                 825                 830
Thr His Val Ser Glu Asp Cys Phe Pro Leu Leu Asp Gly Cys Arg Lys
        835                 840                 845
Asn Arg Gln Lys Trp Gln Ala Leu Ala Glu Gln Gln Glu Lys Met Leu
    850                 855                 860
Ile Asn Gly Glu Ser Gly Gln Ala Lys Arg Asn Trp Val Pro Arg Ala
865                 870                 875                 880
Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly Glu Glu Leu Phe
                885                 890                 895
Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly
            900                 905                 910
His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly
        915                 920                 925
Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro
    930                 935                 940
Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser
945                 950                 955                 960
Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met
                965                 970                 975
Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly
            980                 985                 990
Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val
        995                 1000                1005
Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile
    1010                1015                1020
Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile
1025                1030                1035                1040
Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg
                1045                1050                1055
His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln
            1060                1065                1070
Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr
        1075                1080                1085
Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp
    1090                1095                1100
His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly
1105                1110                1115                1120
Met Asp Glu Leu Tyr Lys
                1125
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<213> Aequorea victoria and human

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Met Ser Trp Ser Pro Ser Leu Thr Thr Gln Thr Cys Gly Ala Trp Glu
1 5 10 15

atg aaa gag cgc ctt ggg aca ggg gga ttt gga aat gtc atc cga tgg 96
Met Lys Glu Arg Leu Gly Thr Gly Gly Phe Gly Asn Val Ile Arg Trp
20 25 30

cac aat cag gaa aca ggt gag cag att gcc atc aag cag tgc cgg cag 144
His Asn Gln Glu Thr Gly Glu Gln Ile Ala Ile Lys Gln Cys Arg Gln
35 40 45

gag ctc agc ccc cgg aac cga gag cgg tgg tgc ctg gag atc cag atc 192
Glu Leu Ser Pro Arg Asn Arg Glu Arg Trp Cys Leu Glu Ile Gln Ile
50 55 60

atg aga agg ctg acc cac ccc aat gtg gtg gct gcc cga gat gtc cct 240
Met Arg Arg Leu Thr His Pro Asn Val Val Ala Ala Arg Asp Val Pro
65 70 75 80

gag ggg atg cag aac ttg gcg ccc aat gac ctg ccc ctg ctg gcc atg 288
Glu Gly Met Gln Asn Leu Ala Pro Asn Asp Leu Pro Leu Leu Ala Met
85 90 95

gag tac tgc caa gga gga gat ctc cgg aag tac ctg aac cag ttt gag 336
Glu Tyr Cys Gln Gly Gly Asp Leu Arg Lys Tyr Leu Asn Gln Phe Glu
100 105 110

aac tgc tgt ggt ctg cgg gaa ggt gcc atc ctc acc ttg ctg agt gac 384
Asn Cys Cys Gly Leu Arg Glu Gly Ala Ile Leu Thr Leu Leu Ser Asp
115 120 125

att gcc tct gcg ctt aga tac ctt cat gaa aac aga atc atc cat cgg 432
Ile Ala Ser Ala Leu Arg Tyr Leu His Glu Asn Arg Ile Ile His Arg
130 135 140

gat cta aag cca gaa aac atc gtc ctg cag caa gga gaa cag agg tta 480
Asp Leu Lys Pro Glu Asn Ile Val Leu Gln Gln Gly Glu Gln Arg Leu
145 150 155 160

ata cac aaa att att gac cta gga tat gcc aag gag ctg gat cag ggc 528
Ile His Lys Ile Ile Asp Leu Gly Tyr Ala Lys Glu Leu Asp Gln Gly
165 170 175

agt ctt tgc aca tca ttc gtg ggg acc ctg cag tac ctg gcc cca gag 576
Ser Leu Cys Thr Ser Phe Val Gly Thr Leu Gln Tyr Leu Ala Pro Glu
180 185 190

cta ctg gag cag cag aag tac aca gtg acc gtc gac tac tgg agc ttc 624
Leu Leu Glu Gln Gln Lys Tyr Thr Val Thr Val Asp Tyr Trp Ser Phe
195 200 205

ggc acc ctg gcc ttt gag tgc atc acg ggc ttc cgg ccc ttc ctc ccc 672
Gly Thr Leu Ala Phe Glu Cys Ile Thr Gly Phe Arg Pro Phe Leu Pro

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    210                  215                  220

aac tgg cag ccc gtg cag tgg cat tca aaa gtg cgg cag aag agt gag      720
Asn Trp Gln Pro Val Gln Trp His Ser Lys Val Arg Gln Lys Ser Glu
225                 230                 235                 240

gtg gac att gtt gtt agc gaa gac ttg aat gga acg gtg aag ttt tca      768
Val Asp Ile Val Val Ser Glu Asp Leu Asn Gly Thr Val Lys Phe Ser
                245                 250                 255

agc tct tta ccc tac ccc aat aat ctt aac agt gtc ctg gct gag cga      816
Ser Ser Leu Pro Tyr Pro Asn Asn Leu Asn Ser Val Leu Ala Glu Arg
            260                 265                 270

ctg gag aag tgg ctg caa ctg atg ctg atg tgg cac ccc cga cag agg      864
Leu Glu Lys Trp Leu Gln Leu Met Leu Met Trp His Pro Arg Gln Arg
        275                 280                 285

ggc acg gat ccc acg tat ggg ccc aat ggc tgc ttc aag gcc ctg gat      912
Gly Thr Asp Pro Thr Tyr Gly Pro Asn Gly Cys Phe Lys Ala Leu Asp
    290                 295                 300

gac atc tta aac tta aag ctg gtt cat atc ttg aac atg gtc acg ggc      960
Asp Ile Leu Asn Leu Lys Leu Val His Ile Leu Asn Met Val Thr Gly
305                 310                 315                 320

acc atc cac acc tac cct gtg aca gag gat gag agt ctg cag agc ttg     1008
Thr Ile His Thr Tyr Pro Val Thr Glu Asp Glu Ser Leu Gln Ser Leu
                325                 330                 335

aag gcc aga atc caa cag gac acg ggc atc cca gag gag gac cag gag     1056
Lys Ala Arg Ile Gln Gln Asp Thr Gly Ile Pro Glu Glu Asp Gln Glu
            340                 345                 350

ctg ctg cag gaa gcg ggc ctg gcg ttg atc ccc gat aag cct gcc act     1104
Leu Leu Gln Glu Ala Gly Leu Ala Leu Ile Pro Asp Lys Pro Ala Thr
        355                 360                 365

cag tgt att tca gac ggc aag tta aat gag ggc cac aca ttg gac atg     1152
Gln Cys Ile Ser Asp Gly Lys Leu Asn Glu Gly His Thr Leu Asp Met
    370                 375                 380

gat ctt gtt ttt ctc ttt gac aac agt aaa atc acc tat gag act cag     1200
Asp Leu Val Phe Leu Phe Asp Asn Ser Lys Ile Thr Tyr Glu Thr Gln
385                 390                 395                 400

atc tcc cca cgg ccc caa cct gaa agt gtc agc tgt atc ctt caa gag     1248
Ile Ser Pro Arg Pro Gln Pro Glu Ser Val Ser Cys Ile Leu Gln Glu
                405                 410                 415

ccc aag agg aat ctc gcc ttc ttc cag ctg agg aag gtg tgg ggc cag     1296
Pro Lys Arg Asn Leu Ala Phe Phe Gln Leu Arg Lys Val Trp Gly Gln
            420                 425                 430

gtc tgg cac agc atc cag acc ctg aag gaa gat tgc aac cgg ctg cag     1344
Val Trp His Ser Ile Gln Thr Leu Lys Glu Asp Cys Asn Arg Leu Gln
        435                 440                 445

cag gga cag cga gcc gcc atg atg aat ctc ctc cga aac aac agc tgc     1392
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Gln Gly Gln Arg Ala Ala Met Met Asn Leu Leu Arg Asn Asn Ser Cys
450 455 460

ctc tcc aaa atg aag aat tcc atg gct tcc atg tct cag cag ctc aag 1440
Leu Ser Lys Met Lys Asn Ser Met Ala Ser Met Ser Gln Gln Leu Lys
465 470 475 480

gcc aag ttg gat ttc ttc aaa acc agc atc cag att gac ctg gag aag 1488
Ala Lys Leu Asp Phe Phe Lys Thr Ser Ile Gln Ile Asp Leu Glu Lys
485 490 495

tac agc gag caa acc gag ttt ggg atc aca tca gat aaa ctg ctg ctg 1536
Tyr Ser Glu Gln Thr Glu Phe Gly Ile Thr Ser Asp Lys Leu Leu Leu
500 505 510

gcc tgg agg gaa atg gag cag gct gtg gag ctc tgt ggg cgg gag aac 1584
Ala Trp Arg Glu Met Glu Gln Ala Val Glu Leu Cys Gly Arg Glu Asn
515 520 525

gaa gtg aaa ctc ctg gta gaa cgg atg atg gct ctg cag acc gac att 1632
Glu Val Lys Leu Leu Val Glu Arg Met Met Ala Leu Gln Thr Asp Ile
530 535 540

gtg gac tta cag agg agc ccc atg ggc cgg aag cag ggg gga acg ctg 1680
Val Asp Leu Gln Arg Ser Pro Met Gly Arg Lys Gln Gly Gly Thr Leu
545 550 555 560

gac gac cta gag gag caa gca agg gag ctg tac agg aga cta agg gaa 1728
Asp Asp Leu Glu Glu Gln Ala Arg Glu Leu Tyr Arg Arg Leu Arg Glu
565 570 575

aaa cct cga gac cag cga act gag ggt gac agt cag gaa atg gta cgg 1776
Lys Pro Arg Asp Gln Arg Thr Glu Gly Asp Ser Gln Glu Met Val Arg
580 585 590

ctg ctg ctt cag gca att cag agc ttc gag aag aaa gtg cga gtg atc 1824
Leu Leu Leu Gln Ala Ile Gln Ser Phe Glu Lys Lys Val Arg Val Ile
595 600 605

tat acg cag ctc agt aaa act gtg gtt tgc aag cag aag gcg ctg gaa 1872
Tyr Thr Gln Leu Ser Lys Thr Val Val Cys Lys Gln Lys Ala Leu Glu
610 615 620

ctg ttg ccc aag gtg gaa gag gtg gtg agc tta atg aat gag gat gag 1920
Leu Leu Pro Lys Val Glu Glu Val Val Ser Leu Met Asn Glu Asp Glu
625 630 635 640

aag act gtt gtc cgg ctg cag gag aag cgg cag aag gag ctc tgg aat 1968
Lys Thr Val Val Arg Leu Gln Glu Lys Arg Gln Lys Glu Leu Trp Asn
645 650 655

ctc ctg aag att gct tgt agc aag gtc cgt ggt cct gtc agt gga agc 2016
Leu Leu Lys Ile Ala Cys Ser Lys Val Arg Gly Pro Val Ser Gly Ser
660 665 670

ccg gat agc atg aat gcc tct cga ctt agc cag cct ggg cag ctg atg 2064
Pro Asp Ser Met Asn Ala Ser Arg Leu Ser Gln Pro Gly Gln Leu Met
675 680 685

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tct cag ccc tcc acg gcc tcc aac agc tta cct gag cca gcc aag aag      2112
Ser Gln Pro Ser Thr Ala Ser Asn Ser Leu Pro Glu Pro Ala Lys Lys
    690                 695                 700

agt gaa gaa ctg gtg gct gaa gca cat aac ctc tgc acc ctg cta gaa      2160
Ser Glu Glu Leu Val Ala Glu Ala His Asn Leu Cys Thr Leu Leu Glu
705                 710                 715                 720

aat gcc ata cag gac act gtg agg gaa caa gac cag agt ttc acg gcc      2208
Asn Ala Ile Gln Asp Thr Val Arg Glu Gln Asp Gln Ser Phe Thr Ala
                725                 730                 735

cta gac tgg agc tgg tta cag acg gaa gaa gaa gag cac agc tgc ctg      2256
Leu Asp Trp Ser Trp Leu Gln Thr Glu Glu Glu Glu His Ser Cys Leu
            740                 745                 750

gag cag gcc tca tgg gta ccg cgg gcc cgg gat cca ccg gtc gcc acc      2304
Glu Gln Ala Ser Trp Val Pro Arg Ala Arg Asp Pro Pro Val Ala Thr
        755                 760                 765

atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg      2352
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
    770                 775                 780

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc      2400
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
785                 790                 795                 800

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc      2448
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
                805                 810                 815

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc      2496
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
            820                 825                 830

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag      2544
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
        835                 840                 845

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag      2592
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
    850                 855                 860

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag      2640
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
865                 870                 875                 880

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc      2688
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
                885                 890                 895

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac      2736
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
            900                 905                 910

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac      2784
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
        915                 920                 925
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ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc 2832
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
930 935 940

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc 2880
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
945 950 955 960

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg 2928
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
965 970 975

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc 2976
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
980 985 990

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag taa 3024
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys *
995 1000 1005

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<212> PRT
<213> Aequorea victoria and human

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1 5 10 15
Met Lys Glu Arg Leu Gly Thr Gly Gly Phe Gly Asn Val Ile Arg Trp
20 25 30
His Asn Gln Glu Thr Gly Glu Gln Ile Ala Ile Lys Gln Cys Arg Gln
35 40 45
Glu Leu Ser Pro Arg Asn Arg Glu Arg Trp Cys Leu Glu Ile Gln Ile
50 55 60
Met Arg Arg Leu Thr His Pro Asn Val Val Ala Ala Arg Asp Val Pro
65 70 75 80
Glu Gly Met Gln Asn Leu Ala Pro Asn Asp Leu Pro Leu Leu Ala Met
85 90 95
Glu Tyr Cys Gln Gly Gly Asp Leu Arg Lys Tyr Leu Asn Gln Phe Glu
100 105 110
Asn Cys Cys Gly Leu Arg Glu Gly Ala Ile Leu Thr Leu Leu Ser Asp
115 120 125
Ile Ala Ser Ala Leu Arg Tyr Leu His Glu Asn Arg Ile Ile His Arg
130 135 140
Asp Leu Lys Pro Glu Asn Ile Val Leu Gln Gln Gly Glu Gln Arg Leu
145 150 155 160
Ile His Lys Ile Ile Asp Leu Gly Tyr Ala Lys Glu Leu Asp Gln Gly
165 170 175
Ser Leu Cys Thr Ser Phe Val Gly Thr Leu Gln Tyr Leu Ala Pro Glu
180 185 190
Leu Leu Glu Gln Gln Lys Tyr Thr Val Thr Val Asp Tyr Trp Ser Phe
195 200 205
Gly Thr Leu Ala Phe Glu Cys Ile Thr Gly Phe Arg Pro Phe Leu Pro
210 215 220
Asn Trp Gln Pro Val Gln Trp His Ser Lys Val Arg Gln Lys Ser Glu
225 230 235 240
Val Asp Ile Val Val Ser Glu Asp Leu Asn Gly Thr Val Lys Phe Ser

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                245                     250                     255
Ser Ser Leu Pro Tyr Pro Asn Asn Leu Asn Ser Val Leu Ala Glu Arg
            260                     265                     270
Leu Glu Lys Trp Leu Gln Leu Met Leu Met Trp His Pro Arg Gln Arg
        275                     280                     285
Gly Thr Asp Pro Thr Tyr Gly Pro Asn Gly Cys Phe Lys Ala Leu Asp
    290                     295                     300
Asp Ile Leu Asn Leu Lys Leu Val His Ile Leu Asn Met Val Thr Gly
305                     310                     315                     320
Thr Ile His Thr Tyr Pro Val Thr Glu Asp Glu Ser Leu Gln Ser Leu
                325                     330                     335
Lys Ala Arg Ile Gln Gln Asp Thr Gly Ile Pro Glu Glu Asp Gln Glu
            340                     345                     350
Leu Leu Gln Glu Ala Gly Leu Ala Leu Ile Pro Asp Lys Pro Ala Thr
        355                     360                     365
Gln Cys Ile Ser Asp Gly Lys Leu Asn Glu Gly His Thr Leu Asp Met
    370                     375                     380
Asp Leu Val Phe Leu Phe Asp Asn Ser Lys Ile Thr Tyr Glu Thr Gln
385                     390                     395                     400
Ile Ser Pro Arg Pro Gln Pro Glu Ser Val Ser Cys Ile Leu Gln Glu
                405                     410                     415
Pro Lys Arg Asn Leu Ala Phe Phe Gln Leu Arg Lys Val Trp Gly Gln
            420                     425                     430
Val Trp His Ser Ile Gln Thr Leu Lys Glu Asp Cys Asn Arg Leu Gln
        435                     440                     445
Gln Gly Gln Arg Ala Ala Met Met Asn Leu Leu Arg Asn Asn Ser Cys
    450                     455                     460
Leu Ser Lys Met Lys Asn Ser Met Ala Ser Met Ser Gln Gln Leu Lys
465                     470                     475                     480
Ala Lys Leu Asp Phe Phe Lys Thr Ser Ile Gln Ile Asp Leu Glu Lys
                485                     490                     495
Tyr Ser Glu Gln Thr Glu Phe Gly Ile Thr Ser Asp Lys Leu Leu Leu
            500                     505                     510
Ala Trp Arg Glu Met Glu Gln Ala Val Glu Leu Cys Gly Arg Glu Asn
        515                     520                     525
Glu Val Lys Leu Leu Val Glu Arg Met Met Ala Leu Gln Thr Asp Ile
    530                     535                     540
Val Asp Leu Gln Arg Ser Pro Met Gly Arg Lys Gln Gly Gly Thr Leu
545                     550                     555                     560
Asp Asp Leu Glu Glu Gln Ala Arg Glu Leu Tyr Arg Arg Leu Arg Glu
                565                     570                     575
Lys Pro Arg Asp Gln Arg Thr Glu Gly Asp Ser Gln Glu Met Val Arg
            580                     585                     590
Leu Leu Leu Gln Ala Ile Gln Ser Phe Glu Lys Lys Val Arg Val Ile
        595                     600                     605
Tyr Thr Gln Leu Ser Lys Thr Val Val Cys Lys Gln Lys Ala Leu Glu
    610                     615                     620
Leu Leu Pro Lys Val Glu Glu Val Val Ser Leu Met Asn Glu Asp Glu
625                     630                     635                     640
Lys Thr Val Val Arg Leu Gln Glu Lys Arg Gln Lys Glu Leu Trp Asn
                645                     650                     655
Leu Leu Lys Ile Ala Cys Ser Lys Val Arg Gly Pro Val Ser Gly Ser
            660                     665                     670
Pro Asp Ser Met Asn Ala Ser Arg Leu Ser Gln Pro Gly Gln Leu Met
        675                     680                     685
Ser Gln Pro Ser Thr Ala Ser Asn Ser Leu Pro Glu Pro Ala Lys Lys
    690                     695                     700
Ser Glu Glu Leu Val Ala Glu Ala His Asn Leu Cys Thr Leu Leu Glu
705                     710                     715                     720
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Asn Ala Ile Gln Asp Thr Val Arg Glu Gln Asp Gln Ser Phe Thr Ala
                725                 730                 735
Leu Asp Trp Ser Trp Leu Gln Thr Glu Glu Glu Glu His Ser Cys Leu
            740                 745                 750
Glu Gln Ala Ser Trp Val Pro Arg Ala Arg Asp Pro Pro Val Ala Thr
        755                 760                 765
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
    770                 775                 780
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
785                 790                 795                 800
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
                805                 810                 815
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
            820                 825                 830
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
        835                 840                 845
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
    850                 855                 860
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
865                 870                 875                 880
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
                885                 890                 895
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
            900                 905                 910
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
        915                 920                 925
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
    930                 935                 940
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
945                 950                 955                 960
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
                965                 970                 975
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
            980                 985                 990
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
        995                 1000                1005
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<210> 11
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<213> Aequorea victoria and human

<220>
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Met Asp Glu Leu Phe Pro Leu Ile Phe Pro Ala Glu Pro Ala Gln Ala
 1               5                  10                  15

tct ggc ccc tat gtg gag atc att gag cag ccc aag cag cgg ggc atg      96
Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln Pro Lys Gln Arg Gly Met
            20                  25                  30

cgc ttc cgc tac aag tgc gag ggg cgc tcc gcg ggc agc atc cca ggc     144
Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser Ala Gly Ser Ile Pro Gly
        35                  40                  45
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gag agg agc aca gat acc acc aag acc cac ccc acc atc aag atc aat 192
Glu Arg Ser Thr Asp Thr Thr Lys Thr His Pro Thr Ile Lys Ile Asn
50 55 60

ggc tac aca gga cca ggg aca gtg cgc atc tcc ctg gtc acc aag gac 240
Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile Ser Leu Val Thr Lys Asp
65 70 75 80

cct cct cac cgg cct cac ccc cac gag ctt gta gga aag gac tgc cgg 288
Pro Pro His Arg Pro His Pro His Glu Leu Val Gly Lys Asp Cys Arg
85 90 95

gat ggc ttc tat gag gct gag ctc tgc ccg gac cgc tgc atc cac agt 336
Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro Asp Arg Cys Ile His Ser
100 105 110

ttc cag aac ctg gga atc cag tgt gtg aag aag cgg gac ctg gag cag 384
Phe Gln Asn Leu Gly Ile Gln Cys Val Lys Lys Arg Asp Leu Glu Gln
115 120 125

gct atc agt cag cgc atc cag acc aac aac aac ccc ttc caa gtt cct 432
Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn Asn Pro Phe Gln Val Pro
130 135 140

ata gaa gag cag cgt ggg gac tac gac ctg aat gct gtg cgg ctc tgc 480
Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu Asn Ala Val Arg Leu Cys
145 150 155 160

ttc cag gtg aca gtg cgg gac cca tca ggc agg ccc ctc cgc ctg ccg 528
Phe Gln Val Thr Val Arg Asp Pro Ser Gly Arg Pro Leu Arg Leu Pro
165 170 175

cct gtc ctt cct cat ccc atc ttt gac aat cgt gcc ccc aac act gcc 576
Pro Val Leu Pro His Pro Ile Phe Asp Asn Arg Ala Pro Asn Thr Ala
180 185 190

gag ctc aag atc tgc cga gtg aac cga aac tct ggc agc tgc ctc ggt 624
Glu Leu Lys Ile Cys Arg Val Asn Arg Asn Ser Gly Ser Cys Leu Gly
195 200 205

ggg gat gag atc ttc cta ctg tgt gac aag gtg cag aaa gag gac att 672
Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys Val Gln Lys Glu Asp Ile
210 215 220

gag gtg tat ttc acg gga cca ggc tgg gag gcc cga ggc tcc ttt tcg 720
Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu Ala Arg Gly Ser Phe Ser
225 230 235 240

caa gct gat gtg cac cga caa gtg gcc att gtg ttc cgg acc cct ccc 768
Gln Ala Asp Val His Arg Gln Val Ala Ile Val Phe Arg Thr Pro Pro
245 250 255

tac gca gac ccc agc ctg cag gct cct gtg cgt gtc tcc atg cag ctg 816
Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val Arg Val Ser Met Gln Leu
260 265 270

cgg cgg cct tcc gac cgg gag ctc agt gag ccc atg gaa ttc cag tac 864
Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu Pro Met Glu Phe Gln Tyr
275 280 285

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ctg cca gat aca gac gat cgt cac cgg att gag gag aaa cgt aaa agg       912
Leu Pro Asp Thr Asp Asp Arg His Arg Ile Glu Glu Lys Arg Lys Arg
    290                 295                 300

aca tat gag acc ttc aag agc atc atg aag aag agt cct ttc agc gga       960
Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys Lys Ser Pro Phe Ser Gly
305                 310                 315                 320

ccc acc gac ccc cgg cct cca cct cga cgc att gct gtg cct tcc cgc      1008
Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg Ile Ala Val Pro Ser Arg
                325                 330                 335

agc tca gct tct gtc ccc aag cca gca ccc cag ccc tat ccc ttt acg      1056
Ser Ser Ala Ser Val Pro Lys Pro Ala Pro Gln Pro Tyr Pro Phe Thr
            340                 345                 350

tca tcc ctg agc acc atc aac tat gat gag ttt ccc acc atg gtg ttt      1104
Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu Phe Pro Thr Met Val Phe
        355                 360                 365

cct tct ggg cag atc agc cag gcc tcg gcc ttg gcc ccg gcc cct ccc      1152
Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala Leu Ala Pro Ala Pro Pro
    370                 375                 380

caa gtc ctg ccc cag gct cca gcc cct gcc cct gct cca gcc atg gta      1200
Gln Val Leu Pro Gln Ala Pro Ala Pro Ala Pro Ala Pro Ala Met Val
385                 390                 395                 400

tca gct ctg gcc cag gcc cca gcc cct gtc cca gtc cta gcc cca ggc      1248
Ser Ala Leu Ala Gln Ala Pro Ala Pro Val Pro Val Leu Ala Pro Gly
                405                 410                 415

cct cct cag gct gtg gcc cca cct gcc ccc aag ccc acc cag gct ggg      1296
Pro Pro Gln Ala Val Ala Pro Pro Ala Pro Lys Pro Thr Gln Ala Gly
            420                 425                 430

gaa gga acg ctg tca gag gcc ctg ctg cag ctg cag ttt gat gat gaa      1344
Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln Leu Gln Phe Asp Asp Glu
        435                 440                 445

gac ctg ggg gcc ttg ctt ggc aac agc aca gac cca gct gtg ttc aca      1392
Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr Asp Pro Ala Val Phe Thr
    450                 455                 460

gac ctg gca tcc gtc gac aac tcc gag ttt cag cag ctg ctg aac cag      1440
Asp Leu Ala Ser Val Asp Asn Ser Glu Phe Gln Gln Leu Leu Asn Gln
465                 470                 475                 480

ggc ata cct gtg gcc ccc cac aca act gag ccc atg ctg atg gag tac      1488
Gly Ile Pro Val Ala Pro His Thr Thr Glu Pro Met Leu Met Glu Tyr
                485                 490                 495

cct gag gct ata act cgc cta gtg aca ggg gcc cag agg ccc ccc gac      1536
Pro Glu Ala Ile Thr Arg Leu Val Thr Gly Ala Gln Arg Pro Pro Asp
            500                 505                 510

cca gct cct gct cca ctg ggg gcc ccg ggg ctc ccc aat ggc ctc ctt      1584
Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly Leu Pro Asn Gly Leu Leu
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515 520 525

tca gga gat gaa gac ttc tcc tcc att gcg gac atg gac ttc tca gcc 1632
Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala Asp Met Asp Phe Ser Ala
530 535 540

ctg ctg agt cag atc agc tcc aag ctt cga att ctg cag tcg acg gta 1680
Leu Leu Ser Gln Ile Ser Ser Lys Leu Arg Ile Leu Gln Ser Thr Val
545 550 555 560

ccg cgg gcc cgg gat cca ccg gtc gcc acc atg gtg agc aag ggc gag 1728
Pro Arg Ala Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly Glu
565 570 575

gag ctg ttc acc ggg gtg gtg ccc atc ctg gtc gag ctg gac ggc gac 1776
Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp
580 585 590

gta aac ggc cac aag ttc agc gtg tcc ggc gag ggc gag ggc gat gcc 1824
Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala
595 600 605

acc tac ggc aag ctg acc ctg aag ttc atc tgc acc acc ggc aag ctg 1872
Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu
610 615 620

ccc gtg ccc tgg ccc acc ctc gtg acc acc ctg acc tac ggc gtg cag 1920
Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln
625 630 635 640

tgc ttc agc cgc tac ccc gac cac atg aag cag cac gac ttc ttc aag 1968
Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys
645 650 655

tcc gcc atg ccc gaa ggc tac gtc cag gag cgc acc atc ttc ttc aag 2016
Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys
660 665 670

gac gac ggc aac tac aag acc cgc gcc gag gtg aag ttc gag ggc gac 2064
Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp
675 680 685

acc ctg gtg aac cgc atc gag ctg aag ggc atc gac ttc aag gag gac 2112
Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp
690 695 700

ggc aac atc ctg ggg cac aag ctg gag tac aac tac aac agc cac aac 2160
Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn
705 710 715 720

gtc tat atc atg gcc gac aag cag aag aac ggc atc aag gtg aac ttc 2208
Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe
725 730 735

aag atc cgc cac aac atc gag gac ggc agc gtg cag ctc gcc gac cac 2256
Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His
740 745 750

tac cag cag aac acc ccc atc ggc gac ggc ccc gtg ctg ctg ccc gac 2304

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Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp
        755                 760                 765

aac cac tac ctg agc acc cag tcc gcc ctg agc aaa gac ccc aac gag      2352
Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu
    770                 775                 780

aag cgc gat cac atg gtc ctg ctg gag ttc gtg acc gcc gcc ggg atc      2400
Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile
785                 790                 795                 800

act ctc ggc atg gac gag ctg tac aag taa                              2430
Thr Leu Gly Met Asp Glu Leu Tyr Lys  *
                805
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Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser Ala Gly Ser Ile Pro Gly
        35                  40                  45
Glu Arg Ser Thr Asp Thr Thr Lys Thr His Pro Thr Ile Lys Ile Asn
    50                  55                  60
Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile Ser Leu Val Thr Lys Asp
65                  70                  75                  80
Pro Pro His Arg Pro His Pro His Glu Leu Val Gly Lys Asp Cys Arg
                85                  90                  95
Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro Asp Arg Cys Ile His Ser
            100                 105                 110
Phe Gln Asn Leu Gly Ile Gln Cys Val Lys Lys Arg Asp Leu Glu Gln
        115                 120                 125
Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn Asn Pro Phe Gln Val Pro
    130                 135                 140
Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu Asn Ala Val Arg Leu Cys
145                 150                 155                 160
Phe Gln Val Thr Val Arg Asp Pro Ser Gly Arg Pro Leu Arg Leu Pro
                165                 170                 175
Pro Val Leu Pro His Pro Ile Phe Asp Asn Arg Ala Pro Asn Thr Ala
            180                 185                 190
Glu Leu Lys Ile Cys Arg Val Asn Arg Asn Ser Gly Ser Cys Leu Gly
        195                 200                 205
Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys Val Gln Lys Glu Asp Ile
    210                 215                 220
Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu Ala Arg Gly Ser Phe Ser
225                 230                 235                 240
Gln Ala Asp Val His Arg Gln Val Ala Ile Val Phe Arg Thr Pro Pro
                245                 250                 255
Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val Arg Val Ser Met Gln Leu
            260                 265                 270
Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu Pro Met Glu Phe Gln Tyr
        275                 280                 285
Leu Pro Asp Thr Asp Asp Arg His Arg Ile Glu Glu Lys Arg Lys Arg
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    290                 295                 300
Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys Lys Ser Pro Phe Ser Gly
305                 310                 315                 320
Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg Ile Ala Val Pro Ser Arg
                325                 330                 335
Ser Ser Ala Ser Val Pro Lys Pro Ala Pro Gln Pro Tyr Pro Phe Thr
            340                 345                 350
Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu Phe Pro Thr Met Val Phe
        355                 360                 365
Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala Leu Ala Pro Ala Pro Pro
    370                 375                 380
Gln Val Leu Pro Gln Ala Pro Ala Pro Ala Pro Ala Pro Ala Met Val
385                 390                 395                 400
Ser Ala Leu Ala Gln Ala Pro Ala Pro Val Pro Val Leu Ala Pro Gly
                405                 410                 415
Pro Pro Gln Ala Val Ala Pro Pro Ala Pro Lys Pro Thr Gln Ala Gly
            420                 425                 430
Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln Leu Gln Phe Asp Asp Glu
        435                 440                 445
Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr Asp Pro Ala Val Phe Thr
    450                 455                 460
Asp Leu Ala Ser Val Asp Asn Ser Glu Phe Gln Gln Leu Leu Asn Gln
465                 470                 475                 480
Gly Ile Pro Val Ala Pro His Thr Thr Glu Pro Met Leu Met Glu Tyr
                485                 490                 495
Pro Glu Ala Ile Thr Arg Leu Val Thr Gly Ala Gln Arg Pro Pro Asp
            500                 505                 510
Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly Leu Pro Asn Gly Leu Leu
        515                 520                 525
Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala Asp Met Asp Phe Ser Ala
    530                 535                 540
Leu Leu Ser Gln Ile Ser Ser Lys Leu Arg Ile Leu Gln Ser Thr Val
545                 550                 555                 560
Pro Arg Ala Arg Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly Glu
                565                 570                 575
Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp
            580                 585                 590
Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala
        595                 600                 605
Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu
    610                 615                 620
Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln
625                 630                 635                 640
Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys
                645                 650                 655
Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys
            660                 665                 670
Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp
        675                 680                 685
Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp
    690                 695                 700
Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn
705                 710                 715                 720
Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe
                725                 730                 735
Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His
            740                 745                 750
Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp
        755                 760                 765
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Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu
    770                 775                 780
Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile
785                 790                 795                 800
Thr Leu Gly Met Asp Glu Leu Tyr Lys
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 1               5                  10                  15

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc      96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20                  25                  30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc     144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc     192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag     240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 65                  70                  75                  80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag     288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag     336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc     384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac     432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac     480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc     528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
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165 170 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc 576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg 624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc 672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc 720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

gga ctc aga tct cga gct caa gct tac atg agc tgg tca cct tcc ctg 768
Gly Leu Arg Ser Arg Ala Gln Ala Tyr Met Ser Trp Ser Pro Ser Leu
245 250 255

aca acg cag aca tgt ggg gcc tgg gaa atg aaa gag cgc ctt ggg aca 816
Thr Thr Gln Thr Cys Gly Ala Trp Glu Met Lys Glu Arg Leu Gly Thr
260 265 270

ggg gga ttt gga aat gtc atc cga tgg cac aat cag gaa aca ggt gag 864
Gly Gly Phe Gly Asn Val Ile Arg Trp His Asn Gln Glu Thr Gly Glu
275 280 285

cag att gcc atc aag cag tgc cgg cag gag ctc agc ccc cgg aac cga 912
Gln Ile Ala Ile Lys Gln Cys Arg Gln Glu Leu Ser Pro Arg Asn Arg
290 295 300

gag cgg tgg tgc ctg gag atc cag atc atg aga agg ctg acc cac ccc 960
Glu Arg Trp Cys Leu Glu Ile Gln Ile Met Arg Arg Leu Thr His Pro
305 310 315 320

aat gtg gtg gct gcc cga gat gtc cct gag ggg atg cag aac ttg gcg 1008
Asn Val Val Ala Ala Arg Asp Val Pro Glu Gly Met Gln Asn Leu Ala
325 330 335

ccc aat gac ctg ccc ctg ctg gcc atg gag tac tgc caa gga gga gat 1056
Pro Asn Asp Leu Pro Leu Leu Ala Met Glu Tyr Cys Gln Gly Gly Asp
340 345 350

ctc cgg aag tac ctg aac cag ttt gag aac tgc tgt ggt ctg cgg gaa 1104
Leu Arg Lys Tyr Leu Asn Gln Phe Glu Asn Cys Cys Gly Leu Arg Glu
355 360 365

ggt gcc atc ctc acc ttg ctg agt gac att gcc tct gcg ctt aga tac 1152
Gly Ala Ile Leu Thr Leu Leu Ser Asp Ile Ala Ser Ala Leu Arg Tyr
370 375 380

ctt cat gaa aac aga atc atc cat cgg gat cta aag cca gaa aac atc 1200
Leu His Glu Asn Arg Ile Ile His Arg Asp Leu Lys Pro Glu Asn Ile
385 390 395 400

gtc ctg cag caa gga gaa cag agg tta ata cac aaa att att gac cta 1248

Val Leu Gln Gln Gly Glu Gln Arg Leu Ile His Lys Ile Ile Asp Leu
405 410 415

gga tat gcc aag gag ctg gat cag ggc agt ctt tgc aca tca ttc gtg 1296
Gly Tyr Ala Lys Glu Leu Asp Gln Gly Ser Leu Cys Thr Ser Phe Val
420 425 430

ggg acc ctg cag tac ctg gcc cca gag cta ctg gag cag cag aag tac 1344
Gly Thr Leu Gln Tyr Leu Ala Pro Glu Leu Leu Glu Gln Gln Lys Tyr
435 440 445

aca gtg acc gtc gac tac tgg agc ttc ggc acc ctg gcc ttt gag tgc 1392
Thr Val Thr Val Asp Tyr Trp Ser Phe Gly Thr Leu Ala Phe Glu Cys
450 455 460

atc acg ggc ttc cgg ccc ttc ctc ccc aac tgg cag ccc gtg cag tgg 1440
Ile Thr Gly Phe Arg Pro Phe Leu Pro Asn Trp Gln Pro Val Gln Trp
465 470 475 480

cat tca aaa gtg cgg cag aag agt gag gtg gac att gtt gtt agc gaa 1488
His Ser Lys Val Arg Gln Lys Ser Glu Val Asp Ile Val Val Ser Glu
485 490 495

gac ttg aat gga acg gtg aag ttt tca agc tct tta ccc tac ccc aat 1536
Asp Leu Asn Gly Thr Val Lys Phe Ser Ser Ser Leu Pro Tyr Pro Asn
500 505 510

aat ctt aac agt gtc ctg gct gag cga ctg gag aag tgg ctg caa ctg 1584
Asn Leu Asn Ser Val Leu Ala Glu Arg Leu Glu Lys Trp Leu Gln Leu
515 520 525

atg ctg atg tgg cac ccc cga cag agg ggc acg gat ccc acg tat ggg 1632
Met Leu Met Trp His Pro Arg Gln Arg Gly Thr Asp Pro Thr Tyr Gly
530 535 540

ccc aat ggc tgc ttc aag gcc ctg gat gac atc tta aac tta aag ctg 1680
Pro Asn Gly Cys Phe Lys Ala Leu Asp Asp Ile Leu Asn Leu Lys Leu
545 550 555 560

gtt cat atc ttg aac atg gtc acg ggc acc atc cac acc tac cct gtg 1728
Val His Ile Leu Asn Met Val Thr Gly Thr Ile His Thr Tyr Pro Val
565 570 575

aca gag gat gag agt ctg cag agc ttg aag gcc aga atc caa cag gac 1776
Thr Glu Asp Glu Ser Leu Gln Ser Leu Lys Ala Arg Ile Gln Gln Asp
580 585 590

acg ggc atc cca gag gag gac cag gag ctg ctg cag gaa gcg ggc ctg 1824
Thr Gly Ile Pro Glu Glu Asp Gln Glu Leu Leu Gln Glu Ala Gly Leu
595 600 605

gcg ttg atc ccc gat aag cct gcc act cag tgt att tca gac ggc aag 1872
Ala Leu Ile Pro Asp Lys Pro Ala Thr Gln Cys Ile Ser Asp Gly Lys
610 615 620

tta aat gag ggc cac aca ttg gac atg gat ctt gtt ttt ctc ttt gac 1920
Leu Asn Glu Gly His Thr Leu Asp Met Asp Leu Val Phe Leu Phe Asp
625 630 635 640

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aac agt aaa atc acc tat gag act cag atc tcc cca cgg ccc caa cct      1968
Asn Ser Lys Ile Thr Tyr Glu Thr Gln Ile Ser Pro Arg Pro Gln Pro
                645                 650                 655

gaa agt gtc agc tgt atc ctt caa gag ccc aag agg aat ctc gcc ttc      2016
Glu Ser Val Ser Cys Ile Leu Gln Glu Pro Lys Arg Asn Leu Ala Phe
            660                 665                 670

ttc cag ctg agg aag gtg tgg ggc cag gtc tgg cac agc atc cag acc      2064
Phe Gln Leu Arg Lys Val Trp Gly Gln Val Trp His Ser Ile Gln Thr
        675                 680                 685

ctg aag gaa gat tgc aac cgg ctg cag cag gga cag cga gcc gcc atg      2112
Leu Lys Glu Asp Cys Asn Arg Leu Gln Gln Gly Gln Arg Ala Ala Met
    690                 695                 700

atg aat ctc ctc cga aac aac agc tgc ctc tcc aaa atg aag aat tcc      2160
Met Asn Leu Leu Arg Asn Asn Ser Cys Leu Ser Lys Met Lys Asn Ser
705                 710                 715                 720

atg gct tcc atg tct cag cag ctc aag gcc aag ttg gat ttc ttc aaa      2208
Met Ala Ser Met Ser Gln Gln Leu Lys Ala Lys Leu Asp Phe Phe Lys
                725                 730                 735

acc agc atc cag att gac ctg gag aag tac agc gag caa acc gag ttt      2256
Thr Ser Ile Gln Ile Asp Leu Glu Lys Tyr Ser Glu Gln Thr Glu Phe
            740                 745                 750

ggg atc aca tca gat aaa ctg ctg ctg gcc tgg agg gaa atg gag cag      2304
Gly Ile Thr Ser Asp Lys Leu Leu Leu Ala Trp Arg Glu Met Glu Gln
        755                 760                 765

gct gtg gag ctc tgt ggg cgg gag aac gaa gtg aaa ctc ctg gta gaa      2352
Ala Val Glu Leu Cys Gly Arg Glu Asn Glu Val Lys Leu Leu Val Glu
    770                 775                 780

cgg atg atg gct ctg cag acc gac att gtg gac tta cag agg agc ccc      2400
Arg Met Met Ala Leu Gln Thr Asp Ile Val Asp Leu Gln Arg Ser Pro
785                 790                 795                 800

atg ggc cgg aag cag ggg gga acg ctg gac gac cta gag gag caa gca      2448
Met Gly Arg Lys Gln Gly Gly Thr Leu Asp Asp Leu Glu Glu Gln Ala
                805                 810                 815

agg gag ctg tac agg aga cta agg gaa aaa cct cga gac cag cga act      2496
Arg Glu Leu Tyr Arg Arg Leu Arg Glu Lys Pro Arg Asp Gln Arg Thr
            820                 825                 830

gag ggt gac agt cag gaa atg gta cgg ctg ctg ctt cag gca att cag      2544
Glu Gly Asp Ser Gln Glu Met Val Arg Leu Leu Leu Gln Ala Ile Gln
        835                 840                 845

agc ttc gag aag aaa gtg cga gtg atc tat acg cag ctc agt aaa act      2592
Ser Phe Glu Lys Lys Val Arg Val Ile Tyr Thr Gln Leu Ser Lys Thr
    850                 855                 860

gtg gtt tgc aag cag aag gcg ctg gaa ctg ttg ccc aag gtg gaa gag      2640
Val Val Cys Lys Gln Lys Ala Leu Glu Leu Leu Pro Lys Val Glu Glu
865                 870                 875                 880
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gtg gtg agc tta atg aat gag gat gag aag act gtt gtc cgg ctg cag      2688
Val Val Ser Leu Met Asn Glu Asp Glu Lys Thr Val Val Arg Leu Gln
                885                     890                     895

gag aag cgg cag aag gag ctc tgg aat ctc ctg aag att gct tgt agc      2736
Glu Lys Arg Gln Lys Glu Leu Trp Asn Leu Leu Lys Ile Ala Cys Ser
            900                     905                     910

aag gtc cgt ggt cct gtc agt gga agc ccg gat agc atg aat gcc tct      2784
Lys Val Arg Gly Pro Val Ser Gly Ser Pro Asp Ser Met Asn Ala Ser
        915                     920                     925

cga ctt agc cag cct ggg cag ctg atg tct cag ccc tcc acg gcc tcc      2832
Arg Leu Ser Gln Pro Gly Gln Leu Met Ser Gln Pro Ser Thr Ala Ser
    930                     935                     940

aac agc tta cct gag cca gcc aag aag agt gaa gaa ctg gtg gct gaa      2880
Asn Ser Leu Pro Glu Pro Ala Lys Lys Ser Glu Glu Leu Val Ala Glu
945                     950                     955                     960

gca cat aac ctc tgc acc ctg cta gaa aat gcc ata cag gac act gtg      2928
Ala His Asn Leu Cys Thr Leu Leu Glu Asn Ala Ile Gln Asp Thr Val
                965                     970                     975

agg gaa caa gac cag agt ttc acg gcc cta gac tgg agc tgg tta cag      2976
Arg Glu Gln Asp Gln Ser Phe Thr Ala Leu Asp Trp Ser Trp Leu Gln
            980                     985                     990

acg gaa gaa gaa gag cac agc tgc ctg gag cag gcc tca tga              3018
Thr Glu Glu Glu Glu His Ser Cys Leu Glu Gln Ala Ser  *
        995                     1000                    1005


&lt;210&gt; 14
&lt;211&gt; 1005
&lt;212&gt; PRT
&lt;213&gt; Aequorea victoria and human

&lt;400&gt; 14
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1               5                  10                  15
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20                  25                  30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
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145                 150                 155                 160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240
Gly Leu Arg Ser Arg Ala Gln Ala Tyr Met Ser Trp Ser Pro Ser Leu
                245                 250                 255
Thr Thr Gln Thr Cys Gly Ala Trp Glu Met Lys Glu Arg Leu Gly Thr
            260                 265                 270
Gly Gly Phe Gly Asn Val Ile Arg Trp His Asn Gln Glu Thr Gly Glu
        275                 280                 285
Gln Ile Ala Ile Lys Gln Cys Arg Gln Glu Leu Ser Pro Arg Asn Arg
    290                 295                 300
Glu Arg Trp Cys Leu Glu Ile Gln Ile Met Arg Arg Leu Thr His Pro
305                 310                 315                 320
Asn Val Val Ala Ala Arg Asp Val Pro Glu Gly Met Gln Asn Leu Ala
                325                 330                 335
Pro Asn Asp Leu Pro Leu Leu Ala Met Glu Tyr Cys Gln Gly Gly Asp
            340                 345                 350
Leu Arg Lys Tyr Leu Asn Gln Phe Glu Asn Cys Cys Gly Leu Arg Glu
        355                 360                 365
Gly Ala Ile Leu Thr Leu Leu Ser Asp Ile Ala Ser Ala Leu Arg Tyr
    370                 375                 380
Leu His Glu Asn Arg Ile Ile His Arg Asp Leu Lys Pro Glu Asn Ile
385                 390                 395                 400
Val Leu Gln Gln Gly Glu Gln Arg Leu Ile His Lys Ile Ile Asp Leu
                405                 410                 415
Gly Tyr Ala Lys Glu Leu Asp Gln Gly Ser Leu Cys Thr Ser Phe Val
            420                 425                 430
Gly Thr Leu Gln Tyr Leu Ala Pro Glu Leu Leu Glu Gln Gln Lys Tyr
        435                 440                 445
Thr Val Thr Val Asp Tyr Trp Ser Phe Gly Thr Leu Ala Phe Glu Cys
    450                 455                 460
Ile Thr Gly Phe Arg Pro Phe Leu Pro Asn Trp Gln Pro Val Gln Trp
465                 470                 475                 480
His Ser Lys Val Arg Gln Lys Ser Glu Val Asp Ile Val Val Ser Glu
                485                 490                 495
Asp Leu Asn Gly Thr Val Lys Phe Ser Ser Ser Leu Pro Tyr Pro Asn
            500                 505                 510
Asn Leu Asn Ser Val Leu Ala Glu Arg Leu Glu Lys Trp Leu Gln Leu
        515                 520                 525
Met Leu Met Trp His Pro Arg Gln Arg Gly Thr Asp Pro Thr Tyr Gly
    530                 535                 540
Pro Asn Gly Cys Phe Lys Ala Leu Asp Asp Ile Leu Asn Leu Lys Leu
545                 550                 555                 560
Val His Ile Leu Asn Met Val Thr Gly Thr Ile His Thr Tyr Pro Val
                565                 570                 575
Thr Glu Asp Glu Ser Leu Gln Ser Leu Lys Ala Arg Ile Gln Gln Asp
            580                 585                 590
Thr Gly Ile Pro Glu Glu Asp Gln Glu Leu Leu Gln Glu Ala Gly Leu
        595                 600                 605
Ala Leu Ile Pro Asp Lys Pro Ala Thr Gln Cys Ile Ser Asp Gly Lys
    610                 615                 620
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Leu Asn Glu Gly His Thr Leu Asp Met Asp Leu Val Phe Leu Phe Asp
625                 630                 635                 640
Asn Ser Lys Ile Thr Tyr Glu Thr Gln Ile Ser Pro Arg Pro Gln Pro
                645                 650                 655
Glu Ser Val Ser Cys Ile Leu Gln Glu Pro Lys Arg Asn Leu Ala Phe
            660                 665                 670
Phe Gln Leu Arg Lys Val Trp Gly Gln Val Trp His Ser Ile Gln Thr
        675                 680                 685
Leu Lys Glu Asp Cys Asn Arg Leu Gln Gln Gly Gln Arg Ala Ala Met
    690                 695                 700
Met Asn Leu Leu Arg Asn Asn Ser Cys Leu Ser Lys Met Lys Asn Ser
705                 710                 715                 720
Met Ala Ser Met Ser Gln Gln Leu Lys Ala Lys Leu Asp Phe Phe Lys
                725                 730                 735
Thr Ser Ile Gln Ile Asp Leu Glu Lys Tyr Ser Glu Gln Thr Glu Phe
            740                 745                 750
Gly Ile Thr Ser Asp Lys Leu Leu Leu Ala Trp Arg Glu Met Glu Gln
        755                 760                 765
Ala Val Glu Leu Cys Gly Arg Glu Asn Glu Val Lys Leu Leu Val Glu
    770                 775                 780
Arg Met Met Ala Leu Gln Thr Asp Ile Val Asp Leu Gln Arg Ser Pro
785                 790                 795                 800
Met Gly Arg Lys Gln Gly Gly Thr Leu Asp Asp Leu Glu Glu Gln Ala
                805                 810                 815
Arg Glu Leu Tyr Arg Arg Leu Arg Glu Lys Pro Arg Asp Gln Arg Thr
            820                 825                 830
Glu Gly Asp Ser Gln Glu Met Val Arg Leu Leu Leu Gln Ala Ile Gln
        835                 840                 845
Ser Phe Glu Lys Lys Val Arg Val Ile Tyr Thr Gln Leu Ser Lys Thr
    850                 855                 860
Val Val Cys Lys Gln Lys Ala Leu Glu Leu Leu Pro Lys Val Glu Glu
865                 870                 875                 880
Val Val Ser Leu Met Asn Glu Asp Glu Lys Thr Val Val Arg Leu Gln
                885                 890                 895
Glu Lys Arg Gln Lys Glu Leu Trp Asn Leu Leu Lys Ile Ala Cys Ser
            900                 905                 910
Lys Val Arg Gly Pro Val Ser Gly Ser Pro Asp Ser Met Asn Ala Ser
        915                 920                 925
Arg Leu Ser Gln Pro Gly Gln Leu Met Ser Gln Pro Ser Thr Ala Ser
    930                 935                 940
Asn Ser Leu Pro Glu Pro Ala Lys Lys Ser Glu Glu Leu Val Ala Glu
945                 950                 955                 960
Ala His Asn Leu Cys Thr Leu Leu Glu Asn Ala Ile Gln Asp Thr Val
                965                 970                 975
Arg Glu Gln Asp Gln Ser Phe Thr Ala Leu Asp Trp Ser Trp Leu Gln
            980                 985                 990
Thr Glu Glu Glu Glu His Ser Cys Leu Glu Gln Ala Ser
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gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc 96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc 144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc 192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag 240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag 288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag 336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc 384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac 432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac 480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc 528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc 576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg 624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc 672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc 720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

gga ctc aga tct cga gct caa gct tcc acc atg atg aat ctc ctc cga 768
Gly Leu Arg Ser Arg Ala Gln Ala Ser Thr Met Met Asn Leu Leu Arg
245 250 255

aac aac agc tgc ctc tcc aaa atg aag aat tcc atg gct tcc atg tct 816
Asn Asn Ser Cys Leu Ser Lys Met Lys Asn Ser Met Ala Ser Met Ser
260 265 270

cag cag ctc aag gcc aag ttg gat ttc ttc aaa acc agc atc cag att 864
Gln Gln Leu Lys Ala Lys Leu Asp Phe Phe Lys Thr Ser Ile Gln Ile
275 280 285

gac ctg gag aag tac agc gag caa acc gag ttt ggg atc aca tca gat 912
Asp Leu Glu Lys Tyr Ser Glu Gln Thr Glu Phe Gly Ile Thr Ser Asp
290 295 300

aaa ctg ctg ctg gcc tgg agg gaa atg gag cag gct gtg gag ctc tgt 960
Lys Leu Leu Leu Ala Trp Arg Glu Met Glu Gln Ala Val Glu Leu Cys
305 310 315 320

ggg cgg gag aac gaa gtg aaa ctc ctg gta gaa cgg atg atg gct ctg 1008
Gly Arg Glu Asn Glu Val Lys Leu Leu Val Glu Arg Met Met Ala Leu
325 330 335

cag acc gac att gtg gac tta cag agg agc ccc atg ggc cgg aag cag 1056
Gln Thr Asp Ile Val Asp Leu Gln Arg Ser Pro Met Gly Arg Lys Gln
340 345 350

ggg gga acg ctg gac gac cta gag gag caa gca agg gag ctg tac agg 1104
Gly Gly Thr Leu Asp Asp Leu Glu Glu Gln Ala Arg Glu Leu Tyr Arg
355 360 365

aga cta agg gaa aaa cct cga gac cag cga act gag ggt gac agt cag 1152
Arg Leu Arg Glu Lys Pro Arg Asp Gln Arg Thr Glu Gly Asp Ser Gln
370 375 380

gaa atg gta cgg ctg ctg ctt cag gca att cag agc ttc gag aag aaa 1200
Glu Met Val Arg Leu Leu Leu Gln Ala Ile Gln Ser Phe Glu Lys Lys
385 390 395 400

gtg cga gtg atc tat acg cag ctc agt aaa act gtg gtt tgc aag cag 1248
Val Arg Val Ile Tyr Thr Gln Leu Ser Lys Thr Val Val Cys Lys Gln
405 410 415

aag gcg ctg gaa ctg ttg ccc aag gtg gaa gag gtg gtg agc tta atg 1296
Lys Ala Leu Glu Leu Leu Pro Lys Val Glu Glu Val Val Ser Leu Met
420 425 430

aat gag gat gag aag act gtt gtc cgg ctg cag gag aag cgg cag aag 1344
Asn Glu Asp Glu Lys Thr Val Val Arg Leu Gln Glu Lys Arg Gln Lys
435 440 445

gag ctc tgg aat ctc ctg aag att gct tgt agc aag gtc cgt ggt cct 1392
Glu Leu Trp Asn Leu Leu Lys Ile Ala Cys Ser Lys Val Arg Gly Pro
450 455 460

gtc agt gga agc ccg gat agc atg aat gcc tct cga ctt agc cag cct 1440
Val Ser Gly Ser Pro Asp Ser Met Asn Ala Ser Arg Leu Ser Gln Pro

465 470 475 480

ggg cag ctg atg tct cag ccc tcc acg gcc tcc aac agc tta cct gag 1488
Gly Gln Leu Met Ser Gln Pro Ser Thr Ala Ser Asn Ser Leu Pro Glu
485 490 495

cca gcc aag aag agt gaa gaa ctg gtg gct gaa gca cat aac ctc tgc 1536
Pro Ala Lys Lys Ser Glu Glu Leu Val Ala Glu Ala His Asn Leu Cys
500 505 510

acc ctg cta gaa aat gcc ata cag gac act gtg agg gaa caa gac cag 1584
Thr Leu Leu Glu Asn Ala Ile Gln Asp Thr Val Arg Glu Gln Asp Gln
515 520 525

agt ttc acg gcc cta gac tgg agc tgg tta cag acg gaa gaa gaa gag 1632
Ser Phe Thr Ala Leu Asp Trp Ser Trp Leu Gln Thr Glu Glu Glu Glu
530 535 540

cac agc tgc ctg gag cag gcc tca tga 1659
His Ser Cys Leu Glu Gln Ala Ser *
545 550

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<212> PRT
<213> Aequorea victoria and human

<400> 16
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20 25 30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

Gly Leu Arg Ser Arg Ala Gln Ala Ser Thr Met Met Asn Leu Leu Arg
245 250 255

Asn Asn Ser Cys Leu Ser Lys Met Lys Asn Ser Met Ala Ser Met Ser
260 265 270

Gln Gln Leu Lys Ala Lys Leu Asp Phe Phe Lys Thr Ser Ile Gln Ile
275 280 285

Asp Leu Glu Lys Tyr Ser Glu Gln Thr Glu Phe Gly Ile Thr Ser Asp
290 295 300

Lys Leu Leu Leu Ala Trp Arg Glu Met Glu Gln Ala Val Glu Leu Cys
305 310 315 320

Gly Arg Glu Asn Glu Val Lys Leu Leu Val Glu Arg Met Met Ala Leu
325 330 335

Gln Thr Asp Ile Val Asp Leu Gln Arg Ser Pro Met Gly Arg Lys Gln
340 345 350

Gly Gly Thr Leu Asp Asp Leu Glu Glu Gln Ala Arg Glu Leu Tyr Arg
355 360 365

Arg Leu Arg Glu Lys Pro Arg Asp Gln Arg Thr Glu Gly Asp Ser Gln
370 375 380

Glu Met Val Arg Leu Leu Leu Gln Ala Ile Gln Ser Phe Glu Lys Lys
385 390 395 400

Val Arg Val Ile Tyr Thr Gln Leu Ser Lys Thr Val Val Cys Lys Gln
405 410 415

Lys Ala Leu Glu Leu Leu Pro Lys Val Glu Glu Val Val Ser Leu Met
420 425 430

Asn Glu Asp Glu Lys Thr Val Val Arg Leu Gln Glu Lys Arg Gln Lys
435 440 445

Glu Leu Trp Asn Leu Leu Lys Ile Ala Cys Ser Lys Val Arg Gly Pro
450 455 460

Val Ser Gly Ser Pro Asp Ser Met Asn Ala Ser Arg Leu Ser Gln Pro
465 470 475 480

Gly Gln Leu Met Ser Gln Pro Ser Thr Ala Ser Asn Ser Leu Pro Glu
485 490 495

Pro Ala Lys Lys Ser Glu Glu Leu Val Ala Glu Ala His Asn Leu Cys
500 505 510

Thr Leu Leu Glu Asn Ala Ile Gln Asp Thr Val Arg Glu Gln Asp Gln
515 520 525

Ser Phe Thr Ala Leu Asp Trp Ser Trp Leu Gln Thr Glu Glu Glu Glu
530 535 540

His Ser Cys Leu Glu Gln Ala Ser
545 550